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SEROTONIN AND VASOSPASM IN SUBARACHNOID HEMORRHAGE

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled: "Serotonin and Vasospasm in Subarachnoid Hemorrhage", submitted by Thomas Wm. Noseworthy in partial fulfillment of the requirements for the degree of Masters of SCIENCE IN EXPERIMENTAL MEDICINE.

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This thesis is dedicated to

my parents, Nellie and Fraser in Newfoundland

and

to my wife Avril Anne and daughter

Kimberley

ABSTRACT

Following human subarachnoid hemorrhage from saccular aneurysms, cerebral arteries may undergo sustained constriction. This cerebral arterial vasospasm represents a major cause of morbidity and mortality in up to one-half of those who sustain subarachnoid hemorrhage. Patients who develop such vasospasm may have transient or permanent ischemic neurological deficits or may die.

The etiology of vasospasm is unknown but many agents have been incriminated. Cerebral arteries are known to be sensitive to the vasoconstrictive properties of serotonin but evidence linking this agent to the disorder remains controversial.

Using a chronic primate model the role of serotonin in the pathogenesis of vasospasm was investigated.

Small and large subarachnoid hemorrhages of autologous blood and platelets were induced in 22 monkeys, which were randomized to receive reserpine and kanamycin, or placebo.

Reserpine interferes with the storage of serotonin within platelet granules causing it to be exposed and thereupon metabolized. Kanamycin alters tryptophan metabolism in the gastrointestinal tract resulting in less serotonin production.

The effect of treatment was assessed for 7 days following subarachnoid hemorrhage. Investigation included angiographic

measurement of vessel caliber, serotonin levels, neurological examination, vital signs and arterial blood gases.

Vasospasm occurred in 77% and 73% of animals at 1 hour and 7 days, respectively, post-subarachnoid hemorrhage. The frequency and severity of vasospasm was equally distributed between treated and control animals whether or not they received a small or large subarachnoid hemorrhage. Treatment invariably lowered serotonin levels but did not lessen vasospasm.

It is concluded that serotonin does not play a central role in the causation of cerebral vasospasm.

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LIST OF ABBREVIATIONS

BOL - 2-bromolysergic acid diethylamine

BP - mean blood pressure

CBF - cerebral blood flow

CSF - cerebrospinal fluid

CT - computed tomography

EDTA - disodium ethylene dinitrito tetra acetate

$EtCO_2$ - End-tidal carbon dioxide

5-HIAA - 5-hydroxyindoleacetic acid

5-HT - 5-hydroxytryptamine

5-HT'ol - 5 hydroxytryptophol

ICP - intracranial pressure

IM - intramuscular

IV - intravenous

KCl - potassium chloride

N_2O_2 - nitrous oxide

O_2 - oxygen

PaO_2 - arterial oxygen tension

$PaCO_2$ - arterial carbon dioxide tension

pH - negative Log of the hydrogen ion concentration, the base 10

RPM - revolution per minute

VSP - vasospasm

ICA - internal carotid artery

MCA - middle carotid artery

PPC - proximal pericallosal artery

DPC - distal pericallosal artery

UNITS OF MEASUREMENT AND DOSAGE

°C - degree centigrade

cm - centimeter

g - gram

kg - kilogram

L - Litre

ug - microgram

mL - milliliter

mg - milligram

mm - millimeters

M - Molar

N - Normal

lb - pound

t.i.d- three times a day

q.i.d- four times daily

CHAPTER 1

VASOSPASM IN SUBARACHNOID HEMORRHAGE

Introduction

Following stimulation by the late Dr. Penfield, Dr. Francis Echlin (1965) decided to investigate the relationship between recurrent focal cerebral ischemia and the production of a focal epileptogenic lesion. During these investigations, he noted that mechanical or electrical stimulation of the pial arteries of the cat caused them to vasoconstrict. He had previously known that larger arteries of the circle of Willis, including the basilar artery, were influenced by the same processes.

In 1951, Eckar and Riemanschneider published an article entitled: "Arteriographic Demonstration of Spasm of the Intracranial Arteries: With Special Reference to Saccular Arterial Aneurysm." Three decades later, it is now widely accepted that cerebral vascular spasm (VSP) may occur in up to one-half the cases of human subarachnoid hemorrhage (SAH) caused by ruptured berry aneurysm (Weir, 1975).

This phenomenon of arterial spasm is a sustained constriction of cerebral blood vessels which are in contact with the blood from the SAH. Resulting from the VSP, brain tissue being supplied by the contracted vessels may develop ischemia or infarction (du Boulay et al, 1980). Transient or

permanent neurological deficits or death may result (Weir, 1975) from this sequence of events.

Though VSP is said to occur following some neurosurgical procedures, ruptured arteriovenous malformations and postconcussive head injury, it is surprisingly uncommon (Adams, 1980; Miller & Gudeman, 1980). Whether this represents a failure to recognize this entity in conditions other than SAH, or whether a unique chain of events occurs following rupture of saccular aneurysms, is unclear.

Ruptured aneurysms, within the circle of Willis, are the commonest cause of human SAH. Population studies report an annual incidence of 7-16.8 cases per 100,000 (Phillips et al, 1980). A retrospective Canadian survey of 274 patients with angiographic or autopsy-proven aneurysms (Wheelock et al, 1983) indicated that VSP of intracranial vessels was a significant associated complication from day 3-12 following the hemorrhage. Angiography carried out postoperatively showed VSP occurring with a variable time course from hours to days.

While a range of incidence from 30-50% of radiographic VSP following aneurysmal rupture has been suggested (Fletcher et al, 1959; Allcock & Drake, 1965), the true frequency of VSP remains unknown, in that there are wide discrepancies in the frequency and timing of cerebral angiography. The incidence of angiographically detectable VSP after SAH depends primarily on the time after SAH at which the angiography is performed (Symon, 1980).

Whatever the true frequency of angiographic VSP following SAH, it has been directly implicated in the sequence of ischemia and infarction that may accompany SAH. Though this is a commonly held notion, the association of measurable cerebral vasoconstriction and cerebral symptomatology raises controversy. Some authors (Millikan, 1975) have suggested that there is no relationship between angiographic VSP and incidence of cerebral infarction as assessed by clinical or postmortem assessments. Though this work has been disputed (Heros, 1976), there remains an accepted discrepancy between cerebral VSP and clinical deterioration, in that some patients demonstrate severe spasm without symptoms. It is likely that there is a critical degree of spasm, variable in different patients under different circumstances, at which cerebral neurological deficits occur. It appears that most investigators and clinicians accept that when VSP is very severe, neurological consequences are more likely. In cases of less marked spasm reliable correlations are not possible.

In 1975, Nibbelink conducted an international cooperative study of aneurysm patients describing cerebral ischemia in at least 25% of patients within the first two weeks of ictus. This correlates well with Japanese work (Saito et al, 1977) showing ischemia in 21% of patients.

Considering that about one-half of patients with ruptured intracranial aneurysms will have angiographically-proven cerebral VSP and 20-25% of patients will show an ischemic

deficit, this is clearly a public health problem demanding attention. Patients, many of whom are in their most productive years, may develop drowsiness, headache, dysphasias, hemiparesis, or even hemiplegia, coma and death. Though recovery may occur in many, it is often gradual and by no means guaranteed. If repeat computed tomography does not demonstrate rebleed in these patients and cerebral angiography demonstrates constricted vessels, it is assumed that many are suffering from VSP induced by SAH.

There have been many factors and agents implicated as the causative or influencing factor for VSP. It remains clear, however, that following rupture of a saccular aneurysm the chain of events ultimately leading to VSP is, in some manner, initiated by the propulsion of intraluminal blood into the subarachnoid space, in the region of the major vessels of the circle of Willis.

Pool (1958) was probably the earliest investigator to implicate a substance from platelets, namely serotonin, as a likely causative agent for VSP. Platelets release serotonin following coagulation (Zucker & Borrelli, 1955). Of all the chemicals known to induce constriction of cerebral vessels serotonin is probably the most powerful vasoconstrictor, exceeding such endogenous agents as angiotensin and various catecholamines (Allen et al, 1974).

The purpose of this thesis is to address the specific issue of serotonin as a potential mediator of cerebral VSP.

Models of subarachnoid hemorrhage and vasospasm

Despite decades of interest in the phenomenon of cerebral arterial VSP associated with SAH there remains, to this date, no animal model which exactly mimics the time course of VSP such as follows SAH in the human. The ideal experimental species for in vivo investigation of VSP would, of course, be Homo sapiens.

Several issues relevent to VSP have confounded investigators interested in developing an analogous animal model. Amongst these questions are:

1. Why is the peak incidence of post-ictal VSP greatest between days 3 - 12 (Weir, 1980)?

2. Why does the human not appear to develop acute VSP within the first few hours after SAH as has been demonstrated in many animal models (Saito & Sano, 1980; Weir, 1980)?

3. Why does there sometimes appear to be a variation in the time course of post-operative VSP, as compared to post-ictal VSP?

4. Why do some patients develop VSP after aneurysm surgery, even though the aneurysm has never bled (Peerless, 1980; Fein 1980) and the operation has occurred in a virtually bloodless field.

5. Why is acute and chronic VSP not as recognized, or appreciated, following other neurological insults causing SAH, such as head injuries (Wilkins, 1980) or tumor surgery resulting in significant subarachnoid blood loss?

Though these questions, and many others, lack definitive answers, it remains clear that any animal model which is intended to duplicate human VSP must be chronic and produce delayed spasm lasting more than a few days. Ideally, the optimal animal model should involve a species with an intracranial circulation that is anatomically, physiologically and pharmacologically similar to the human. The SAH should be produced from vessels having abnormalities similar to intracranial berry aneurysms, VSP should last weeks, be persistent on angiography and result in a focal ischemic neurological deficit. Finally, the animal must survive the insult to receive treatment modalities in prospective, randomized, blinded investigations. Developing such a model remains a difficult task.

In vitro experiments

Progress in our understanding of VSP has taken place in in vitro studies of arterial segments exposed to agents, such as serotonin, believed to induce VSP. These experiments allow accurate measurement of mechanical contractility of vessels induced by carefully controlled administration of vasoactive agonists and antagonists (Okwuasaba et al, 1981).

The current information from these studies, however, cannot be directly extropolated to treat human VSP (Cook, 1984). Removal of biological tissue from its natural habitat creates a man-made environment. Apart from the unknown, unmeasurable quantity of trauma induced by removing these vessels, it is mandatory that their nourishment and longevity during chronic experiments does not alter vascular reactivity.

In vivo experiments

Though patients that have sustained SAH have been investigated in clinical trials (Allen et al, 1983; Zervas et al, 1979) with substances anticipated to attenuate the frequency and severity of VSP, it remains essential that in vivo experiments in animal models continue and that the models improve. To date, animals such as monkeys, baboons, dogs and cats have been subjected to experimentation. Both the anterior circulation with the internal carotid and its branches (Petruk, 1976), as well as posterior circulation including the basilar and its divisions (Zervas et al, 1973), have been examined. Direct observations through an open skull (Echlin, 1965) and carotid angiography in the closed skull (Ritchie, 1979; Espinosa et al, 1984) have provided means of observing VSP.

Angiographically-proven caliber change is a common method of assessing the presence of VSP but does not always correlate with the delayed neurological ischemic deficits in humans

(Zingesser et al, 1968) or animals (Hashi et al, 1972). Consequently, measurement of hemispheral and compartmental cerebral blood flow (CBF) has been used by some investigators (Ferguson et al, 1972) to complement angiographic data. Simeone (1972) has suggested that flow must be decreased to 40% of control, or less, to be significant. Petruk (1973) has shown that angiographic VSP and measureable reduction in CBF are not always coincident, though there is a relationship between the severity of reduced perfusion and the degree of neurological deficit.

A number of spasmogenic agents have been directly applied to cerebral vessels in in vivo experiments, or alternatively, have been injected into cerebral vessels, in order to produce VSP. However, the application of blood or blood products directly upon vessels (Espinosa et al, 1984) or within the subarachnoid space (Noseworthy et al, 1984) has been popular in an attempt to duplicate naturally-occurring SAH. An expansion of this model has been the puncture (Espinosa et al, 1984) or avulsion (Brawley et al, 1968) of an intracranial artery allowing the SAH to occur around the injured vessel.

Though in vivo animal models in conjunction with in vitro experiments have significantly advanced our knowledge of VSP, the best model to mimic the human incidence, onset and course following aneurysm rupture remains to be developed.

The platelet and its relation to vasoactive substances

In 1954, Humphrey reported the importance of platelets as the vehicle for active pharmacological substances which, when released have significant vasoactive effects. The platelets of many species have been shown to contain significant quantities of serotonin, with little present in the plasma unless platelet damage has occurred.

Platelets are known to be short-lived cellular constituents of blood and lack the enzymatic mechanism to synthesize serotonin (Gaddum & Giarman, 1956). Stacey (1961) showed that platelets avidly take up and bind the amine. The storage sites for serotonin are the dense cytoplasmic granules in which it probably exists in a non-diffusible complex.

Zucker (1955) carried out elegant experiments to show that plasma separated from aged or traumatized blood, or platelet-rich plasma, was strongly vasoactive. Alternatively, oxalated plasma separated quickly by centrifugation, serum prepared by recalcification of platelet-poor plasma, and serum from patients with thrombocytopenia (below $20,000/mm^3$) had one-tenth, or less, of the vasoconstrictive activity found in platelet-rich plasma. In an attempt to determine which forms of platelet damage promoted vasoconstrictor liberation, this group carried out experiments involving platelet destruction by mechanical means. If serum with an intact number of washed platelets was compared to a suspension of an equal number of

platelets mechanically disrupted in a mortar with fine sand, there was no significant difference in the low vasoconstrictor activity. Only sonic disruption of platelets, with complete platelet destruction and microscopically-proven disappearance of all intact platelets, released serotonin to the same extent as the coagulation process. Clearly, the results show that during coagulation human platelets release serotonin which then acts as a vasoconstricting substance in the absence of antagonists. As previously noted, Allen (1974; 1976; 1979) showed that serotonin was indeed a potent cerebral vasoconstrictor.

Reserpine and kanamycin

The effect of serotonin on isolated cerebral arteries may be antagonized by a variety of agents (Toda et al, 1976). Other drugs deplete the platelet of serotonin and thereby alter its vasoconstrictive properties (Markwardt, 1968).

It has been suggested that reserpine interferes with the storage (Maynert & Isaac, 1968; Pletscher, 1968) but probably not the uptake within platelet granules. Consequently, the amine is exposed and thereupon metabolized by monamine oxidase in the mitochondria. The serotonin liberated by reserpine is quickly transformed into two metabolites, 5-hydroxyindoleaceticacid (5-HIAA) and 5- hydroxytryptophol (5-HT'ol). Electron microscopy shows the disappearance of dense osmiophilic organelles containing serotonin, following exposure to reserpine.

Rosoff (1971) reported that animals did not develop the sequence of pulmonary arterial spasm, right heart strain and death, associated with acute pulmonary embolism, if they were pre-treated with reserpine. These animals had demonstrable depletion of platelet-born serotonin.

Zervas et al (1973) showed that reserpine lowered the total blood serotonin levels in a canine model and suggested that treatment with this drug "consistently and completely prevented the development of experimental cerebral VSP".

Kanamycin, an antibiotic, used in this group's primate studies (Zervas et al, 1974) is not known to be a specific serotonin antagonist. In fact, in the accumulated literature of at least the last decade, little mention is made of kanamycin having any relation to serotonin. Haverback (1958) showed that platelet serotonin falls sharply after partial removal of the gastrointestinal tract in both animals and humans. The addition of a nonabsorbable antibiotic, such as kanamycin, to the diet of rats appears to accomplish a similar reduction (Rosoff, 1968). Though the precise mechanism of the serotonin-reducing effect of kanamycin remains an enigma, it is probably related to its attenuation of indigenous intestinal microflora responsible for the metabolism of tryptophan to 5-hydroxytryptamine (serotonin).

Zervas et al (1979), extrapolating from the work of Rosoff and Haverback, reasoned that treatment with

intramuscular reserpine and oral kanamycin would reduce serotonin levels and decrease the incidence of cerebral ischemia following rupture of intracranial aneurysms in humans.

Pharmacology of serotonin

Introduction

Serotonin is a fascinating, yet confusing, substance found widely in both the animal and plant kingdoms. This biogenic amine belongs to a group of pharmacological agents known as autacoids, which are substances with intense pharmacological activity, normally present in the body but not known to be hormones or neurohumors. From the pharmacological point of view, serotonin shares its membership in the family of autacoids with other potent endogenous substances such as histamine and angiotensin. Since the discovery of this agent in 1930 by Ersparmer, in Italy, and by Rapport, working independently at the Cleveland Clinic, this indolalkylamine has been the subject of intense research interest and confusion.

The agent was first synthesized in 1948 by Rapport, Green and Page. In 1952 Erspamer established that it was the same substance, "enteramine", previously known to cause intestinal contraction and peristalsis. Once the substance was given an identifiable chemical structure and synthesized, research began with widely divergent areas of interest.

Occurrence in nature

Studies in comparative anatomy suggest that serotonin is present in the brain of all vertebrate classes (Brodie et al, 1962). The brains of reptiles and amphibians have relatively high concentrations in comparison with mammals. Fish, on the other hand, have lower brain concentrations than mammals. The brains of 16 North American reptiles have been analysed for serotonin by Quay and Wilhoft (1964). Interestingly, whole brain content exhibited an inverse relationship to the animal's size, the lowest concentration being found in the alligator's brain.

Amongst the lower animals, serotonin is similarly widespread, being found in various crustaceans (Welsh and Moorhead, 1959) the venom apparatus of Hymenoptera and scorpions (Welsh and Batty, 1963) and the nematocysts of Hydra.

In the plant kingdom serotonin is found in stinging nettles and distributed through such different sources as banana, plantain, plum, pineapple, walnut, avocado, egg plant and tomato (Wier & Tyler, 1963).

Biosynthesis

In 1952 Blaschko suggested that serotonin was synthesized from tryptophan. Tryptophan, an essential amino acid in the diet, is hydroxylated in the fifth position to form 5-

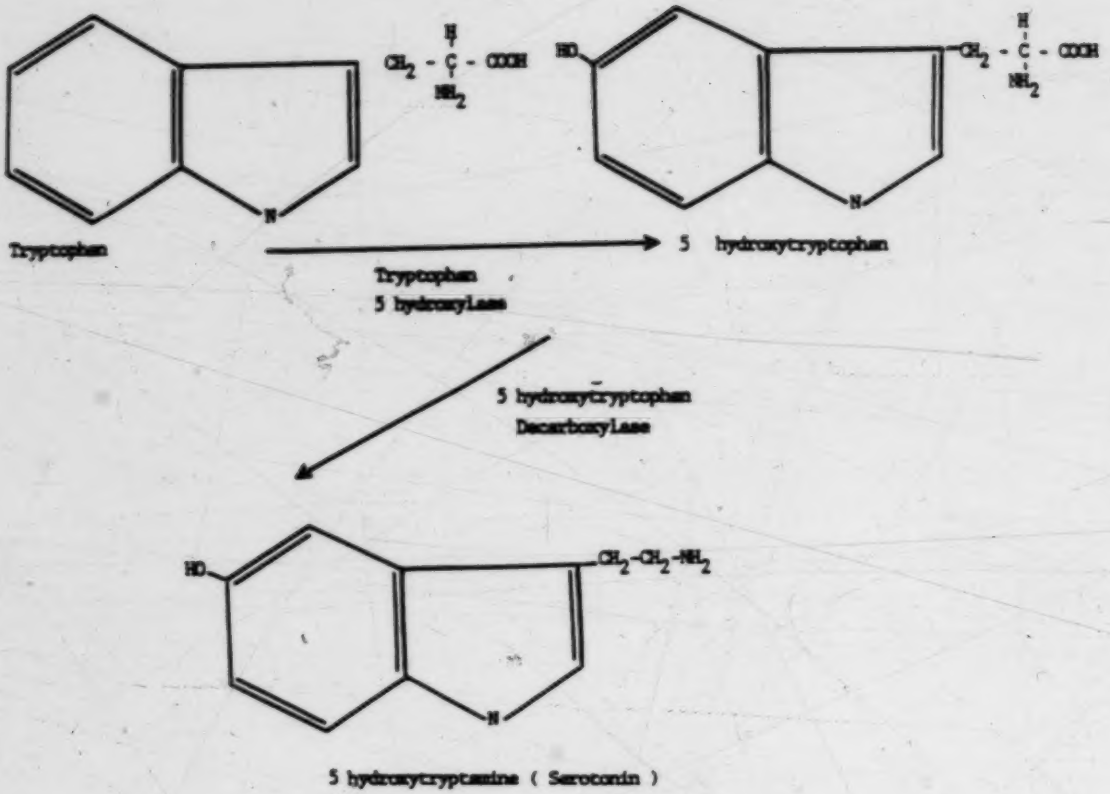
hydroxytryptophan, a substance difficult to identify in blood and tissues because of its low concentration. This intermediate substrate is then altered by a decarboxylase enzyme, which removes the carboxyl group, resulting in 5-hydroxytryptamine, otherwise known as serotonin (Fig 1).

By way of comparison to other enzyme systems, there is an amazing similarity between enzymes responsible for the synthesis and degradation of serotonin as compared to epinephrine. The amino acid precursor of epinephrine is phenylalanine. The step for the production of epinephrine from phenylalanine is hydroxylation in the fifth position.

In man and most mammals the metabolic pathway for production of tryptophan is such that only about 1-2% of the tryptophan is converted to serotonin. Interestingly enough, in carcinoid tumours, malignancies in which there is excessive production of serotonin, biosynthesis is altered in such a way that some 50-60% of dietary tryptophan is converted to serotonin.

Cells either synthesize serotonin, take it up from the blood stream or do both. Some tissues such as bone marrow and platelets do not possess the decarboxylase enzyme necessary for the production of serotonin. Nevertheless, it is widely accepted that platelets contain most of the serotonin in the blood. Presumably, some tissues and organs which are not

Fig. 1: Tryptophan metabolism to 5-hydroxytryptamine (Serotonin)



capable of manufacturing serotonin concentrate the substance from other sources.

Distribution and uptake

There is a wide variation and extent of distribution of serotonin throughout the body. Following the injection of C^{14}-serotonin (Axelrod & Inscoe, 1963), approximately 50% of the agent is metabolized while the remainder is distributed through various tissues of the body such as the reticuloendothelial cells of the spleen and liver, enterochromaffin cells of the intestinal mucosa, platelets, and the lung.

One of the most important sites for removal of circulating serotonin appears to be the lung. It was demonstrated over 50 years ago by Starling and Verney (1925) that the vasoconstrictive activity of the blood disappeared after perfusion through lungs of dogs.

In most storage cells, decarboxylation of 5-hydroxytryptophan occurs within the cytoplasm. The serotonin produced then enters storage granules. The uptake of serotonin is so widespread and variable in different tissues and organs that no clues are provided as to its physiological function (Page, 1968).

Metabolism and inactivation

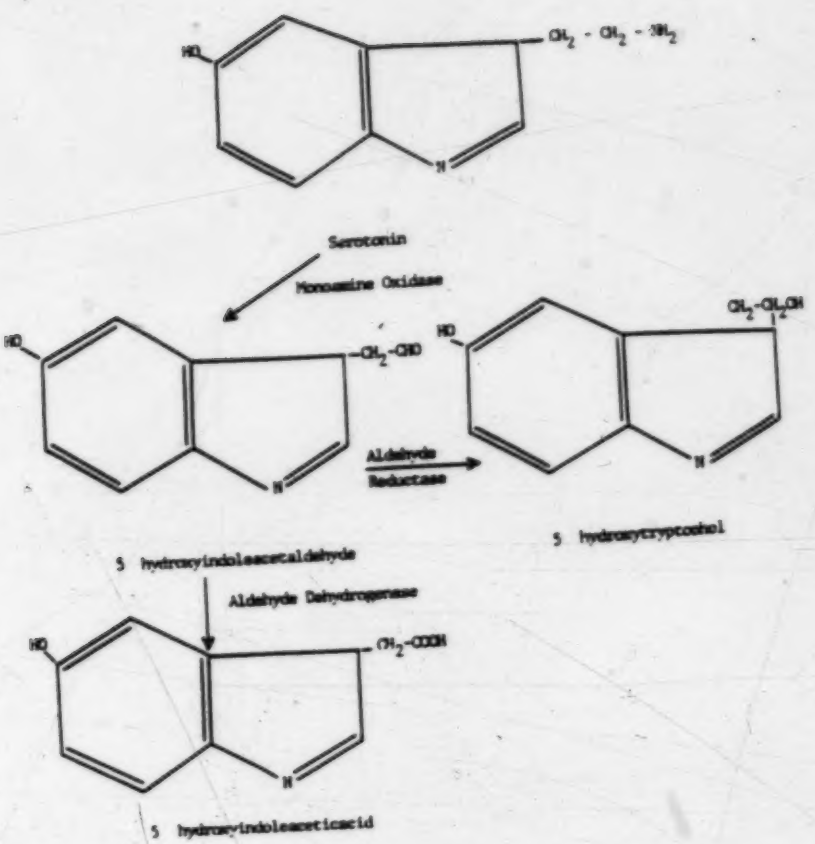
Like epinephrine and other biogenic amines, serotonin is metabolized by the monoamine oxidase enzyme system. In the process of degradation, serotonin is first converted to 5-hydroxyindoleacetaldehyde (Fig 2). As a result of aldehyde dehydrogenase, 5-hydroxyindoleacetaldehyde is converted to 5-hydroxyindoleacetic and 5-hydroxyindoleaceturic acids. These major metabolites are then excreted in the urine.

To a lesser extent, there is evidence for conjugation and acetylation of 5-hydroxytryptamine. A small portion of serotonin is excreted unchanged in the urine (5-9%) (Page, 1968).

Pharmacological effects

The pharmacological activity of serotonin is characteristically unpredictable and variable. Smooth muscle cells of the vasculature, for instance, may develop either intense vasoconstriction or vasodilatation depending upon the particular vessels stimulated by serotonin (Page, 1958). Whereas coronary and skeletal muscle vessels respond with vasodilatation, vessels of the central nervous system, kidney and placenta respond with vasoconstriction.

Fig. 2: Degradation of serotonin



The variability of pharmacological response is further confused by the observation that serotonin can affect physiological parameters in equal yet opposite directions, such as its effects on blood pressure in man. The initial response to serotonin is a fall in blood pressure followed by a pressor response with a marked rise in total systemic vascular resistance. The late effect of serotonin is again vasodilatation in the musculoskeletal bed and a depressor response.

The diversity in pharmacological effects of serotonin is at times bewildering. The very existence of this substance has frequently proven to be a nuisance to mammalian physiologists when conducting perfusion experiments on various body tissues. The following is a brief review of the influence of serotonin on select vascular beds and organs.

cardiovascular system

As previously mentioned, serotonin may evoke vasoconstriction or vasodilatation, depending on which vascular bed is being studied and possibly on the dose. Similar to epinephrine, serotonin demonstrates amphibaric actions with vasoconstriction or vasodilatation. However, epinephrine is much less variable in its response, with low concentrations giving rise to vasodilatation and high concentrations predictably giving rise to vasoconstriction.

If one examines the blood pressure response to the injection of serotonin, the depressor, pressor and late depressor phases can be demonstrated (Page, 1958). The early depressor phase appears to be the result of the Bezold-Jarish reflex (Salmoiraghi et al, 1956). This reflex is also known as the "coronary chemoreflex". It is the result of inhibition of sympathetic outflow and increased cardiac vagal activity leading to profound hypotension associated with bradycardia, as opposed to the usual tachycardia. Vagus section abolishes this effect. The pressor response that follows the depressor phase results from an increase in peripheral vascular resistance and chemoreceptor stimulation. Late depressor phases are attributable to the direct vasodilatory effects in the skeletal muscle. Serotonin appears to raise blood pressure when systemic vascular resistance is low and lower it when the resistance is high. Additionally, arterial blood pressure responses vary widely, depending on the species studied.

At the level of the capillaries, serotonin seems to give rise to increased capillary permeability with the formation of local edema and swelling. This, however, has only been demonstrated in lower order mammals but not in man.

The action of serotonin on the heart may be one of either positive or negative inotropy and chronotropy (Page, 1958). Studies on isolated papillary muscle of cat, dog and rabbit suggest that serotonin does not increase the strength of

contraction. However, in isolated rabbit hearts it increases the rate but not the strength of contraction, while in the dog heart it increases the strength of contraction but slows the rate. In general, serotonin is felt to be a coronary artery vasodilator.

pulmonary circulation

Serotonin is well known as a potent vasoconstrictor of the pulmonary circulation (Dawes & Comroe, 1954). This is important in view of the established diminished reactivity of pulmonary vessels to many recognized vasoconstrictors. Notably, this vasoconstriction exceeds that induced by either epinephrine or norepinephrine and these vascular effects may occur at doses insufficient to effect systemic arterial pressure. The action of serotonin on pulmonary vessels appears to be direct vasoconstriction, which results in subsequent pulmonary hypertension.

As previously discussed, the lung appears to either remove or destroy a large portion of circulating or injected serotonin (Starling & Verney, 1925; Rapport et al, 1948). For decades it has been accepted that an extract of lung has a powerful destructive action on serotonin. It remains a question whether or not pulmonary tissue actually destroys the amine. Mammalian physiologists have perfused blood through lungs to prevent vasoconstriction in other organs perfused by the same blood.

Any physiological role of serotonin in the lung is unknown but it is appreciated that the vascular effects that result from pulmonary thromboembolism may be the result of many vasoactive mediators, with serotonin being a prime candidate. Certainly, Smith (1955) showed that intravenous injection of a clot, formed in vitro, produced a greater circulatory effect than did an embolism of starch granules. Serotonin antagonists diminished the vascular effects of this clot injection.

renal circulation

Alteration of the concentration of serotonin induces variable responses in the renal circulation (Page, 1958). Serotonin has been shown to have an antidiuretic effect (Abrahams and Pickford, 1956; Corcoran et al, 1954). This may be as a result of a change in glomerular filtration, renal plasma flow or tubular reabsorption of water.

If canine kidneys are removed and perfused, Page et al (1953) showed that serotonin causes vasoconstriction. Unlike the pulmonary circulation, however, epinephrine and norepinephrine are more potent renal vasoconstrictors. Whether or not serotonin plays a role in renal hemodynamics in normal or disordered states remains uncertain.

peripheral circulation

As previously cited, Page (1958; 1968) has proposed that serotonin dilates vessels with high neurogenic tone but constricts those with low tone. While serotonin has a strong direct vasoconstricting action on vessels concerned with peripheral resistance, small concentrations of serotonin may actually inhibit neurogenic tone.

In canine foreleg arteries, serotonin causes vasodilation, an effect opposite to that produced by adrenalin and noradrenalin. However, if serotonin is infused into the human brachial artery (Page, 1958) there is a decrease in forearm and hand blood flow. Evidently, the pharmacological effect of serotonin in most vascular beds is unpredictable, related to the infusion concentration, the organ perfused and the species chosen.

cerebral circulation

A significant quantity of information exists to show that serotonin is a potent vasoconstrictor when topically applied to cerebral vessels (Raynor & McMurtry, 1961; Boisvert, 1978). Unlike some other vascular beds, serotonin is not known to induce the opposite effect of vasodilatation of cerebral vessels, at varied concentrations or in different species. The potent vasoconstrictor effect of serotonin has implicated

a causal relationship between serotonin and cerebral VSP following SAH.

The role of serotonin in vasospasm - conflicting evidence

The development of VSP following ruptured aneurysm is preceded by the propulsion of blood into the subarachnoid space. This blood subsequently clots and during this process there is platelet degradation and release of vasoactive mediators such as serotonin. Because cerebral arteries are sensitive to the vasoconstrictive properties of serotonin, this agent has been incriminated as having a role in clinical VSP.

In order for any agent, including serotonin, to be responsible for VSP, the agent must be vasoactive and it, or its metabolites, must be recoverable at the site of SAH. Additionally, VSP should be preventable by either removing the agent or alternatively antagonising its spasmogenic effects. Of particular interest in human cerebral VSP, there has to be an adequate explanation for the time course of development of VSP in relation to the SAH. Does the available evidence suggest that serotonin fulfills these criteria?

Vasoactivity

Raynor, McMurtry and Pool (1961) were the first to suggest that VSP might be due to serotonin. They studied the middle cerebral arteries of 27 cats. Using time-lapse

photomicroscopy the vessels were observed after application of serotonin creatinine sulphate, serum obtained from blood allowed to clot 1-3 days previously and fresh whole blood. None of these experiments continued beyond 3 hours. Application of serotonin to the arteries resulted in marked spasm of a 1-2 cm segment at the point of exposure, the vessels constricting to 20-40% of their original size. Serum from blood previously allowed to clot caused VSP comparable to that of serotonin, while freshly drawn blood had no spasmogenic effect.

Using in vitro arterial strips of cerebral and peripheral canine arteries, Toda et al (1973) demonstrated a transition occurring from the characteristic responses seen in mesenteric arteries (high sensitivity to norepinephrine, low sensitivity to serotonin) to those in cerebral arteries (high sensitivity to serotonin, low sensitivity to norepinephrine). In fact, he showed that serotonin was by far the most potent cerebral vasoconstrictor.

Starling et al (1975) isolated human basilar arteries to study with vasoactive substances and showed them to be more sensitive to serotonin than noradrenalin. Plasma lacked vasoconstrictive properties as opposed to serum. It is particularly important that responses to serotonin were potentiated by serum. Extrapolating this information to human SAH, cerebral arteries may be more sensitive to the vasoactive

properties of agents such as serotonin, following exposure to serum.

In addition to the in vitro and in vivo experiments noted, angiographic studies have confirmed cerebral VSP induced by subarachnoid blood and serotonin. Chow et al (1968) studied 23 cynomologus monkeys following instillation of fresh autologous blood or serotonin into the cisterna magna. Arterial contraction occurred within 5 minutes of the injection of serotonin, reached a maximum by 90 minutes and had returned to normal by 3 hours. In 10 animals subjected to SAH with 2 ml of fresh autologous blood, arterial spasm was more pronounced than that caused by serotonin. Maximum constriction was noted at 5 minutes postinjection but was less by 3 hours.

The work of Allen et al (1974; 1976; 1979) must be included in any review of evidence for vasoactivity associated with serotonin. He concluded that serotonin is probably the agent in blood responsible for the cerebral arterial spasm following SAH. This conclusion was drawn from in vitro experiments using a small volume chamber to determine the contractile activity of various vasoactive agents on canine basilar and middle cerebral arteries (1974). Serotonin produced a 90% maximum contraction of the arteries at a concentration 10-30 times less than that of blood and was the only agent tested that produced a maximum contraction at a concentration known to be present free in clotted blood.

In further work (1974), again using in vitro experiments, the vasoactivity of human serum and CSF was examined using the canine basilar artery. It was suggested that serotonin produced the majority of contractile activity in CSF samples collected 2-7 days following SAH, when applied to the canine arteries.

Finally, in a third publication (1974) in vivo intracisternal injections of serotonin and blood were performed in dogs. "Physiological" concentrations of serotonin caused cerebral arterial spasm for at least 3 hours, comparable to that produced by injection of blood containing approximately the same amount of serotonin.

As a consequence of these three reported experiments, Allen proposed that spasm was directly related to the amount of free serotonin present in the CSF, and in the clots surrounding cerebral arteries. He reasoned that serotonin was produced over a period of several days as clot lysed and platelets released their vasoactive mediators.

Serotonin could indeed be responsible for the rapid phase of vascular contraction seen in animals. The antagonist, methylsergide appears to prevent this phase. However, Okwuasaba et al (1981) could not show that methylsergide prevented the protracted phase which is more typical of human cerebral VSP. They investigated the effects on canine cerebral vessels repeatedly exposed to serotonin. Basilar

artery preparations became virtually unresponsive after less than 20 minutes exposure.

Sustained vasoconstriction from agonists is not well accepted in the present knowledge of pharmacology (Cook; 1984). Consequently, if serotonin is responsible for chronic VSP it must be postulated that it sets a cascade of events in motion, which results in prolonged VSP not reversed by usual antagonists.

Further challenge to the work of Allen et al occurred in 1979, when Boisvert used in vitro and in vivo experiments to examine the cerebrovascular responses to subarachnoid blood and serotonin in the monkey.

Bioassay of monkey serum showed serotonin concentrations of 5×10^{-6} M following clotting. This value was similar to serum concentrations in human and dogs (Allen et al, 1974). As expected, application of this concentration of stock serotonin to isolated primate cerebral vessels produced near-maximal contraction in all cases. Because cyproheptadine blocked the vasoconstrictive activity of monkey serum, it was assumed the vascular effect was caused by liberated serotonin. In further experiments, SAH induced in the chiasmatic cistern produced a significant, persistent decrease in cerebral vessel caliber which was not relieved by cyproheptadine. Furthermore, subarachnoid injection of serotonin in a concentration of 5×10^{-6} M produced a lack of effect on cerebral blood flow or vessel caliber. This was in conflict

with previous work (Allen, 1974) in which maximum contraction was produced at serotonin concentrations of 5×10^{-7} M. Subarachnoid injections of stock serotonin at concentration 100 times greater than those observed by Allen in the CSF of patients with VSP produced only minor changes in vascular caliber of the monkey.

How does one summarize and reconcile these results? Firstly, it can be agreed that serotonin is a potent vasoconstrictor when topically applied to animal and human cerebral arteries. Secondly, when serotonin is injected into the subarachnoid space in in vivo experiments, dilution within the CSF occurs and not all authors have demonstrated consistent vasoconstriction following the administration of so-called physiological concentrations (i.e. 5×10^{-6} M).

Finally, experiments showing acute VSP associated with serotonin cannot be applied to chronic VSP seen following human SAH. Even if serotonin, at any concentration, causes acute VSP, there is no evidence to date that serotonin, or its metabolites, are present or active 5 to 7 days later.

In 1976, Boullin studied CSF from 34 patients with SAH following ruptured aneurysm. His group used strips of isolated basilar artery and applied serotonin, noradrenalin and 6 prostaglandins to observe vasoactivity. Contractile substances were present from CSF in 16 out of 22 patients with VSP but in only 2 of 12 patients without VSP and none of the samples from a controlled myelography group. The vasoactive

responses were not blocked by the serotonin antagonists methylsergide or 2-bromolysergic acid diethylamine (BOL). Nevertheless, the contractile effect of arterial responses to serotonin was potentiated with prior CSF application to the vessel. The interpretation of these experiments is complicated. One might assume that failure to block vasoactive properties with anti-serotonin drugs mitigate against its involvement in the production of VSP. Alternatively, it could be hypothesized that once "activated" CSF is in contact with vessels, that potentiation of the vasoconstriction caused by serotonin occurs and this promotes and maintains cerebral VSP.

It has been argued that if serotonin is released following SAH and is the mediator for VSP, then it should be measurable in CSF and the level should correlate with the severity of angiographic VSP. Tagari et al (1983), using high performance liquid chromatography, demonstrated serotonin in the peri-aneurysmal CSF obtained at operation from 13 patients with SAH. Voldby et al (1982) studied 26 patients with ruptured aneurysms and measured serotonin concentrations repeatedly by radioimmunoassay. The serotonin level in ventricular CSF collected between the second and fifteenth day after SAH did not differ from the levels found in the CSF of control patients. As well, the serotonin content did not correlate with the severity of angiographic VSP or clinical grade. Despite this, 2 patients with severe postoperative VSP demonstrated a 5-fold increase in serotonin concentration.

Even in patients with severe, diffuse VSP, however, serotonin concentration was often normal or low.

Other authors (Allen et al, 1976; Buckell, 1964) have measured high serotonin concentrations in the hematoma fluid surrounding the aneurysm or the lumbar CSF. The concentrations found fell short of those that have been shown to produce half maximal vasoconstriction in in vitro studies.

Lobato et al (1980) reconciled these findings by analysing the time course of the changes induced by SAH in relation to the sensitivity of feline cerebral arteries to serotonin. Interestingly, cerebral arteries displayed a supersensitivity to the drug, most pronounced 3 days after the SAH, but lasting as long as 4 weeks after the SAH. These results suggest that serotonin, alone or in combination with other factors, might produce the late phase of cerebral VSP. This potentially argues against the need to recover serotonin beyond the first few post-ictal days.

To summarize, many spasmogens may be present in the CSF following SAH and be factors in the production of VSP. Variability appears in the results dealing with the concentration of serotonin required to produce VSP in vitro versus in vivo. Some patients have high serotonin concentration in their CSF or subarachnoid hematomas, while others with demonstrable VSP have normal or low levels. Thus, it is unlikely that serotonin mediates delayed VSP, unless

supersensitivity of vessels following SAH accounts for prolonged cerebral vascular responses.

Treatment - studies which suggest a role for serotonin

The evidence for and against the vasoactive role of serotonin and its presence or absence at the site of SAH has been reviewed and one is left with no firm conclusions. If serotonin is removed, or its actions antagonized, will cerebral VSP be lessened? Because this question addresses the issue of prevention and treatment, it is difficult to extrapolate the clinical value of in vitro studies of serotonin antagonists.

There is no doubt that serotonin can constrict cerebral vessels in vitro. Allen et al (1974) showed that methylsergide, a potent serotonin antagonist, reversibly blocked the canine basilar artery response to serotonin, even though it caused dose-dependent contractions of its own. Samples of blood-containing CSF from patients with a variety of disorders (eg. SAH, subdural hemorrhage and brain tumor) also produce contraction of smooth muscle preparations (Weir et al, 1980). Interestingly though, methylsergide does not significantly diminish the contractions induced by bloody CSF.

Zervas et al (1973) reported a reduction in cerebral VSP by treating dogs with reserpine. These authors suggested that reserpine prevented the storage of platelet serotonin and reduced the blood serotonin content for up to a week.

Arterial VSP was produced in dogs by injecting autologous or homologous blood into the cisterna magna, under direct vision. Vertebral angiography was used to monitor the caliber of the basilar artery. The dogs were divided into 4 groups (Table 1).

Most animals were not studied beyond 2 days. The untreated dogs receiving autologous blood (group 1) developed VSP within 15 minutes, which was maximal by 30 minutes and subsiding by 120 minutes. Only 2 of 9 dogs in this group were followed for up to a week and these were shown to have persistent VSP. Dogs which had been pretreated with reserpine before SAH, given autologous blood (group 2), did not develop comparable degrees of VSP to the untreated animals. The third group of normal dogs injected with homologous reserpinized blood developed significantly less VSP than Group 1, while the fourth group of reserpine pre-treated dogs receiving untreated, homologous blood developed severe vasoconstriction. This paper concluded that donor blood used in the production of SAH, derived from reserpine pre-treated dogs, did not cause significant VSP, while blood from untreated donors caused VSP whether or not the recipient animal was pretreated with reserpine. Because serotonin levels in the SAH injectate were predictably low in groups 2 and 3, this paper suggested that the factor primarily predisposing to VSP was, indeed, serotonin. Although these results did not lead the authors to

Table 1: Canine study treatment designation
(Zervas et al, 1973)

TREATMENT DESIGNATION

CANINE STUDY - ZERVAS

1. Normal, untreated dogs, given untreated autologous blood.

2. Reserpine pre-treated dogs, given autologous blood.

3. Normal, untreated dogs, given homologous blood from reserpine-treated dogs.

4. Reserpine pre-treated dogs, given untreated homologous blood from other dogs.

advocate reserpine as a form of treatment for patients with SAH, this stimulated further research in their laboratory.

Several points should be raised, however. The experiments were limited to only 48 hours post-SAH, providing no opportunity to assess the development or prevention of VSP during the peak period most often seen in humans. Furthermore, it was not possible to decide whether hypotension in reserpine-treated dogs, as opposed to untreated dogs, played a role in determining their vascular caliber. Reserpine, of course, may well have influenced other unmeasured vasoactive amines, in adrenergic neurofibers and ganglia, and thus it is not possible to demonstrate that serotonin played the central role.

The following year the same group reported (Zervas et al, 1974) the experimental inhibition of serotonin by an antibiotic, resulting in the prevention of cerebral VSP in the anterior circulation of rhesus monkeys. Kanamycin treatment was chosen because of some evidence that it might alter tryptophan metabolism in the gut and thus lower serotonin levels. Four groups were studied (Table 2).

Whole blood for serotonin estimation was collected before and after these treatments. Two to 4 weeks later the animals received a SAH induced by puncture of the middle cerebral artery and were followed with cerebral angiography up to 3 hours and again on the second and seventh days. The serotonin

Table 2: Primate studies - treatment designation (Zervas et al, 1974)

TREATMENT DESIGNATION

PRIMATE STUDY- ZERVAS

GROUP 1 - kanamycin treatment, orally (n=6)

GROUP 2 - desoxypyridoxine, a pyridoxine antagonist (n=4)

GROUP 3 - intramuscular reserpine - 0.1 mg/kg (n=4)

GROUP 4 - untreated (n=5)

levels dropped significantly with kanamycin treatment (group 1) equal to or less than 26% of normal in 4 of 6 animals. Desoxypyridoxine (Group 2) was less effective in that levels were reduced to about 55% of normal. The levels were reduced to less than 7% normal in animals treated with reserpine (group 3).

VSP did not occur in any of the reserpine-treated animals but severe VSP occurred in all untreated animals, those receiving desoxypyridoxine and the 2 animals receiving kanamycin that did not have a serotonin level < 50% normal.

This work appeared to suggest that oral kanamycin was effective in preventing cerebral VSP following SAH, only when serotonin levels were significantly reduced. It was concluded that treatment of patients with cerebral aneurysms with reserpine and kanamycin might prove useful in preventing postoperative VSP.

Unfortunately, this paper did not provide data comparing percent change in vessel caliber of untreated monkeys to those treated with reserpine or desoxypyridoxine. It did not make clear the rationale for the use of desoxypyridoxine and most importantly provided no comparative blood pressure or blood gas results for all groups at each time frame.

In 1977 Watts reported his work on the vasoactive effects on canine basilar arteries of autologous blood from dogs

pretreated with reserpine. He divided his 13 animals into 3 groups (Table 3).

His results showed that animals in group 1 failed to develop VSP in response to application of fresh autologous blood, while VSP was seen in all animals in group 2 following application of 5-day old clotted autologous blood. Treatment with methylsergide reversed the VSP in 3 of 6 of these animals, but none of the dogs in group 3 developed VSP following application of reserpinized 5-day old clotted blood. The same conclusions were drawn as previously, namely that serotonin was the primary vasoactive substance and that anti-serotonin drugs, should be investigated as treatment for this condition.

In 1979 Zervas et al did just that. They carried out a randomized trial of reserpine and kanamycin to lower the concentration of serotonin. The incidence of cerebral vascular ischemic complications and death, following the rupture of intracranial aneurysms, was studied in 54 patients. Treated patients received reserpine 0.2 mg subcutaneously q.i.d. and kanamycin 1 g orally t.i.d. up to the date of aneurysmal repair. Patients had angiography for confirmation of aneurysm, usually repeated prior to planned surgery and 1 week postoperatively. Periodic blood serotonin levels and daily clinical grading of patients was assessed. The control group contained 28 patients, the treated group 26. VSP was deemed present when patients with neurological impairment had

Table 3: Canine arteries - vasoactive studies
Group designation (Watts, 1977)

VASOACTIVE EFFECTS - CANINE ARTERIES

-WATTS

GROUP 1 - fresh autologous blood applied for 30 minutes to basilar artery (n=3)

GROUP 2 - 5 day old autologous blood clot applied to basilar artery (n=6). Above procedure postblood irrigation - methylsergide application (n=3)

GROUP 3 - reserpine-treated dogs - 5 day old autologous blood clot applied to basilar artery (n=4)

associated cerebral angiographic vasoconstriction in the absence of rebleeding, hydrocephalus or other causes for neurological impairment. Preoperatively 8 control and 1 treated patient developed symptomatic VSP. Three control and 2 treated patients were added when they developed undiagnosed neurological deterioration which was attributed to VSP. This led to a significant difference in the frequency of clinical VSP between treatment and control patients ($p<0.05$). There was no correlation between the preoperative clinical findings of ischemia and angiographic evidence of VSP.

Postoperatively 4 of 16 control patients developed delayed ischemic complications, 1 of whom died, while 1 of 22 treated patients developed delayed ischemic complications and died. VSP diagnosed on postoperative angiography was said to parallel the clinical findings, nevertheless the report described 8 of 13 control patients with VSP and 3 of 16 treated. On admission, blood serotonin levels were not different between treatment and control patients, whether or not they subsequently developed preoperative ischemic complications or death. As expected, patients treated with reserpine and kanamycin had significantly reduced serotonin levels. Serotonin levels at the time of operation were compared with the subsequent development of VSP in the postoperative period. The author stated: "the data suggests that patients with reduced serotonin levels just prior to operation had minimal or no postoperative VSP." Notably,

however, 3 treated patients did have moderate or severe postoperative VSP. The actual values of preoperative serotonin and the index of postoperative VSP are worth perusal (Table 4 and 5). No statistical anlaysis was included with these values.

Despite this, the Human Studies Committee of Massachusettes General Hospital decided that there was evidence to justify treatment of all patients having SAH with reserpine and kanamycin.

Consequently, an additional 35 nonrandomized patients were treated immediately after admission. After excluding 6 such patients who failed to demonstrate cerebral aneurysms, 3 of the remaining 29 patients developed ischemic signs. Of the 24 patients undergoing operation, 2 developed intraoperative neurological defects and 1 developed postoperative ischemic deterioration.

In summary, this study suggested that reserpine and kanamycin reduced the incidence of cerebral ischemia and infarction associated with reduction of blood serotonin. This conclusion is difficult to accept in view of an absence of statistical analysis comparing pre-operative serotonin levels and postoperative VSP. As well, the inclusion of patients in good neurological condition, without neurological disturbance, may have unfairly biased the results. The treatment used, in many patients, namely 0.2 mg q.i.d. of reserpine and 1 g kanamycin t.i.d. was notably far less than that used in their

Table 4: Human studies - serotonin levels and degree of VSP (Zervas et al, 1979)

SEROTONIN LEVELS AND DEGREE OF VSP

HUMAN STUDY - ZERVAS

CONTROL PATIENTS

Serotonin ug/mg	Index of Postop VSP
.19	3+
.12	2+
.22	4+
.11	1+
NA	4+
.12	4+
NA	4+
.11	1+
.06	1+
.29	3+
.16	4+
NA	0
.09	4+

TREATED PATIENTS

Serotonin ug/ml	Index of Postop VSP
.03	1+
.01	1+
.11	4+
.06	1+
.01	0
.01	2+
.03	1+
.01	2+
.01	0
.16	1+
.01	2+
.07	0
.06	1+
.01	1+
NA	4+
.17	3+

Table 5: Serotonin studies - degree of vasospasm
Summary table (Zervas et al, 1979)

SEROTONIN LEVELS AND DEGREE OF VSP

HUMAN STUDY - ZERVAS

SUMMARY TABLE

CONTROLS		PRETREATED
Index VSP	n	
4+	- 6	4+ - 2
3+	- 2	3+ - 1
2+	- 1	2+ - 3
1+	- 3	1+ - 7
0	- 1	0 - 3

canine and primate studies. If one assumes a mean weight of 60 kg each patient received approximately 0.01 mg/kg reserpine as opposed to 0.1 mg/kg given to dogs. Similarly each patient received approximately 42 mg/kg kanamycin as opposed to 100 mg/kg in monkeys.

Finally, and most importantly, the trial was terminated on the assumption that the evidence that the treatment worked was conclusive. The reported data permits one to question these conclusions.

Treatment - studies which do not suggest a role for serotonin

In 1963 Raynor and McMurtry exposed the middle cerebral artery in 16 cats and serially photographed the size of vessels to determine their size. Various test substances were applied topically to these vessels. Arterial VSP lasted 18 minutes when reserpine was given intravenously to animals whose middle cerebral arteries were exposed to serum from blood clotted 2 hours previously. Serum prepared in the same fashion from animals treated 16 hours previously with reserpine caused local arterial spasm which lasted 6 minutes. Spasm lasted 12-15 minutes, despite reserpine treatment, when the topically-applied serum was 2 1/2 days old. Reserpine, 1-benzyl-2, 5-dimethylbufolenin hydrochloride and chlorpromazine were each used as potential serotonin blockers, but none of these prevented spasm when used before serum application, or reversed it in less than 5 minutes once spasm was present.

An appropriate criticism of this study is the small number of animals used and the very short duration of observation.

Boullin et al (1978) conducted a much more detailed analysis of serotonin blockade using a potent antagonist BW501 C67, Alpha-anilino-N-2-chlorphenoxypropyl-acetamidine HCl monohydrate (agent B). VSP in 11 baboons was studied, in a total of 16 experiments. Following injection of blood into the cisterna magna to produce VSP, agent B failed to influence the time course of VSP in major cerebral vessels. This was in contradistinction to animals in which intracisternal injection of serotonin resulted in VSP, which was reversed by agent B.

When human basilar arteries were exposed to serotonin, the VSP which occurred was reversed by agent B. Most notably, when the arteries were exposed to human serum, or CSF from patients with SAH and associated VSP, the resultant contractions were not reversed by agent B.

In summary, agent B, a potent serotonin antagonist failed to reverse in vitro contraction of human basilar artery produced by human CSF, serum and platelet extracts. Acute cerebral VSP produced in baboons by intracisternal injection of blood was not antagonized, though serotonin-induced VSP was.

Though it is not clear from this paper how many experiments of each type were performed, the results question the role of serotonin in the etiology of human VSP.

Boisvert et al (1979) conducted experiments on the cerebrovascular responses to subarachnoid blood and serotonin in monkeys, one year after Boullin and showed that blood-induced VSP was essentially unaffected by intravenous administration of the serotonin antagonist cyproheptadine. Furthermore, physiological concentrations of serotonin present in normal monkey serum did not induce VSP when injected into the subarachnoid space. Only concentrations 10 times the physiological values produced VSP similar to that produced by blood, though the VSP was of a shorter duration. Besides questioning a role for serotonin in the production of post-SAH VSP, these data reflected concern that in vitro results did not reflect the ability of serotonin to constrict vessels in intact primates.

A clinical series by Knuckey and Stokes (1982) reviewed the medical management of patients in two groups from 1971 - 1976 and 1977 - 1979. Reserpine and kanamycin treatment was used within 3 days of SAH. Twenty-six percent of the retrospective control group and 32% of the reserpine and kanamycin treated group developed VSP, demonstrating no obvious protective effect from preoperative administration of the drugs.

The postoperative effects of reserpine and kanamycin administered preoperatively were assessed in the treatment and control group as well. Of the untreated control patients, 17.2% developed postoperative cerebral VSP compared to 6.3% who were treated. Though these authors concluded that the incidence of postoperative VSP was decreased by preoperative administration of reserpine and kanamycin, only 7 treated patients, as opposed to 45 controlled patients were studied. No statistical analysis was supplied with these data.

Blumenkropf et al (1979) studied 7 patients 1 to 3 days post-SAH and obtained serial levels of serum serotonin, plasma norepinephrine and plasma dopamine beta-hydroxylase, together with angiography on at least 2 occasions. Initially, serotonin levels were elevated in 3 of 7 patients but decreased to, or below, normal with reserpine and kanamycin. Five of 6 patients with ruptured aneurysms developed VSP but there was no correlation between angiographic VSP, serum serotonin, plasma norepinephrine, or dopamine beta-hydroxylase levels. Angiographic VSP was not reduced in patients treated with reserpine and kanamycin. Two patients experiencing neurological deterioration attributable to VSP did so with normal or low serotonin levels.

Other Studies (Robertson, 1980) have failed to show an attenuation of VSP with reserpine and kanamycin therapy.

Table 6 reviews results of experiments from this literature review. In short, no firm conclusions can be

Table 6: Conclusions from literature review

CONCLUSIONS FROM LITERATURE REVIEW

1. Serotonin is a vasoactive substance which, when topically applied, induces vasoconstriction in cerebral vessels.
2. Serotonin antagonists block cerebral VSP induced by the amine but do not predictably block VSP induced by blood or vasospastic CSP.
3. A discrepancy exists between the concentration of serotonin necessary to cause VSP in vivo and more physiological concentrations which have been shown to cause VSP in vitro.
4. Patients are said to have both reduced and unchanged instances of VSP if they are treated pre- or postoperatively with serotonin antagonists.

drawn. Yet, reserpine and kanamycin therapy is being used in clinical settings. It appeared appropriate, therefore, to re-investigate the use of these agents in a chronic primate model.

Chapter 2

EXPERIMENTAL STUDY

Experimental objective and approach

The evidence for and against serotonin as a causative agent for the development of human cerebral VSP following SAH allows one to draw no firm conclusions. Clearly, serotonin constricts both human and animal cerebral vessels. However, the cited clinical studies using various agents known to antagonize the vasoconstriction induced by serotonin have produced conflicting results. Despite this, it is presumed that information gained from in vitro and in vivo experiments demonstrating the vasoconstrictive effect of serotonin (Allen et al, 1974; Zervas et al 1973, 1974), together with the support of clinical studies showing a reduction in cerebral ischemia by serotonin blocking drugs (Zervas et al, 1979) has been, and continues to be, applied in the treatment of patients following SAH. It seemed appropriate in view of the large body of information that did not suggest a major role for serotonin in the production of post-SAH VSP, to try to duplicate the treatment efficacy of anti-serotonin medications in an experimental model.

The choice of a model

Because no ideal animal model existed, a choice of an available and accomplishable compromise had to be made.

Despite the easy availability and low cost of an in vitro model, criticism of the relevance of any conclusions drawn, to the etiology of cerebral VSP, remains a concern.

Note was made of the work of Bouillin (1976) comparing primate and human cerebral arteries and suggesting that both contain serotonin receptors and respond to vasospastic factors in the subarachnoid space and hemorrhage into the CSF. Additionally, both baboons and monkeys were suitable for cerebral angiography and have an intracranial circulation not dissimilar to humans.

Cynomolgus monkeys of 2.5-4.0 Kg which are commercially available are easily handled. They tolerate anaesthesia and surgical intervention relatively well and allow access to the CSF through the chiasmatic cistern. As a model of this type had been successfully utilized by several other previous investigators in this laboratory it was decided to continue with this approach.

Unique aspects of the model

Several factors needed to be considered in the model design:

1. The SAH injectate needed to be potentially rich in serotonin.

2. The animals had to receive anti-serotonin drugs or placebo in a randomized, blinded fashion.

3. The treated animals had to have significant reduction in their measurable serotonin levels in order to assess the impact of the alteration upon resultant cerebral vascular caliber.

4. The animals had to be maintained beyond the immediate post-SAH period to allow for comparative assessment of treatment versus control, thereby partially duplicating the human time course of post-ictal VSP. .

With these factors in mind, the injectate of autologous blood was boosted with a platelet concentrate harvested from the animal just prior to the instillation of the SAH. If vasoactive mediators, such as serotonin, contained within the platelet granules were central to the production of VSP, it would produce an ideal situation in which to investigate anti-serotonin drugs. By using platelets collected just prior to the instillation of the SAH, a greater chance of injecting viable vasoactive platelets was assumed.

The choice of anti-serotonin drugs was made simple by the previous work in which both reserpine and kanamycin were administered to animals (Zervas et al, 1973, 1974) and humans (Zervas et al, 1979). A dose of these drugs was chosen to duplicate the proportional quantity used in human studies and it was assumed that the ultimate ability of these mediators to lower the serotonin levels could be assessed by comparing the

treated and control animals. Bias was minimized by having serotonin results unavailable until the angiographic measurements were completed at the end of the study.

Pretreating animals before SAH and lowering the serotonin levels is not equatable with the human clinical setting, even though Zervas (1973) showed that blood taken from reserpine pre-treated dogs failed to cause canine basilar artery VSP, as opposed to the significant VSP induced by blood from untreated dogs. Consequently, animals did not receive treatment or control medications until after SAH. They received their first medication within 2-4 hours post-SAH. Though medications were administered early, it was felt that this protocol of management would approximate that which might occur in referral centers seeing and treating patients within hours of saccular aneurysm rupture. Finally, to have any relevance to human post-ictal VSP, the animals had to be maintained and treated for at least the duration over which the peak period of human VSP occurs, namely 5-7 days. During this period of time administration of medication had to be ensured and neurological examination was necessary.

The objective

In a chronic primate model of post-SAH VSP, to conduct a randomized, double-blind trial of the efficacy of reserpine and kanamycin to:

- induce reductions in blood serotonin levels.
- establish whether these reductions of blood serotonin levels are associated with a discernable reduction in the frequency or severity of VSP.
- determine if frequency and severity of VSP is influenced by the size of the SAH injectate.
- show the relationship between the Cushing hypertensive response and development fo VSP

Hypotheses

1. Reserpine and kanamycin lower blood serotonin levels.
2. Reductions of blood serotonin levels are not associated with a discernable reduction in frequency and severity of VSP.
3. The frequency and severity of VSP is directly influenced by the size of the SAH.

4. The Cushing response is not related to the occurrence of VSP.

Methods and materials

Animal preparation

Twenty-seven female cynomolgus monkeys (Macaca fascicularis) weighing 2.7 - 4.0 kg were used in the study. To be evaluable animals had to survive 7 days post-SAH, receive all medications and have confirmed SAH at autopsy. Three animals died within 6 hours of SAH induction, 2 others were eliminated once postmortem examination established that the hemorrhage was confined to the subdural space. The remaining 22 monkeys, which were studied for 7 days after induction of SAH are reported.

Measured and observed variables included mean arterial blood pressure (BP), heart rate, arterial blood gases, end-tidal CO_2 ($ETCO_2$), cerebral vessel caliber from carotid angiograms, hematocrit and neurological status. All variables, including carotid angiography, were assessed on Day 0 prior to induction of SAH, at 1 hour post-SAH and at Day 7 (Table 7).

Samples of whole blood for serotonin determinations were collected on Day 0 pre-SAH and Day 7. Serotonin estimations were performed using the method of Yuwiler et al (1970), discussed in detail later. Results were blinded to the experimenter and the assistant measuring the cerebral vascular

Table 7: Protocol for cerebral angiography

ANGIOGRAPHY:

LATERAL & OBLIQUE ANGIOGRAMS - DAY 0 (PRE-SAH)

- DAY 0 (1H POST-SAH)

- DAY 7

VESSELS MEASURED AT 4 FIXED POINTS:

ICA

MCA

PPC

DPC

(MEASURED SIX TIMES - CALCULATED AVERAGE FOR EACH)

calibre. The status of the animal, any treatment and vessel measurements were unknown to the technician performing the serotonin estimations.

Neurological examination was performed daily, with particular emphasis on level of consciousness, pupillary responses to light, corneal reflexes and comparative movement of upper and lower extremities. Postmortem examination was conducted after completing all measurements on day 7.

Anaesthesia was induced with sodium pentobarbital (Nembutal R - Abbott Laboratories, Montreal, P.Q.) 25 to 30 mg/kg IV and maintained with 1:1 $N_2O:O_2$ administered via an orotracheal tube (Portex Blue-line R - Portex Ltd, Wilmington, Mass.) with a ventilator (Harvard Apparatus Inc - Dover Rd., Millis, Mass.) Neuromuscular paralysis was induced and maintained with gallamine (Flaxedil R - Rhone - Poulenc Pharma Inc., Montreal, P.Q.), 2 mg/kg I.V. given every 45 min. Procaine penicillin (Ayercillin R - Ayerst Laboratories, Montreal, P.Q.) 100,000 U IM was administered after anaesthetic induction and at the end of Day 0. Rectal temperature (Yellow Springs Instrument Co. - Yellow Springs, OH.) was measured by an electronic thermometer, and maintained at 37^0C by means of a thermostat-controlled heating pad placed beneath the monkey.

Using an operating microscope (Mentor CM III microscope R - Codman & Shurlett Inc., Randolph, Mass.) the cervical portion of the left common carotid artery was dissected and a

20-gauge teflon catheter (Quik - Cath R - Travenol Laboratories, Deerfield, Mass.) was inserted intraluminally through the space enclosed by a 8-0 silk purse-string suture (Ethicon - Peterborough, Ontario) positioned approximately 3 cm proximal to the bifurcation. The suture was placed in the wall of the common carotid artery, with a diameter of the circle formed by the purse-string of approximately 1 mm. This carotid artery catheter was threaded to within 1 cm proximal to the origin of the internal carotid artery and secured and connected by 2 three-way stopcocks to an automated injector device (Cordis Corp. - Miami, Fl) for carotid angiography. The remaining stopcock port was connected to a pressure transducer (Statham P23Db R - Statham, Puerto Rico) for continuous monitoring and printout of arterial blood pressure and heart rate (Beckman Dynograph recorder R - Beckman, Harbour Boulevard, Fullerton, Ca.)

Arterial blood samples were collected periodically throughout the experiments, to monitor the hematocrit (Model Mass.) and to measure (Micro 13 R - Instrumentation Laboratory, Lexington, Mass.) and maintain pH, $PaCO_2$ and PaO_2 within the physiological range throughout the experiment. Fluctuations in $PaCO_2$ were minimized by continuous monitoring of $ETCO_2$ (Beckman Medical Gas Analyzer LB2 R - Beckman, Harbour Boulevard, Fullerton, Ca.) with adjustments of tidal volume and maintenance of constant respiratory rate, to maintain $ETCO_2$ appropriately 5%.

Preparation of SAH injectate

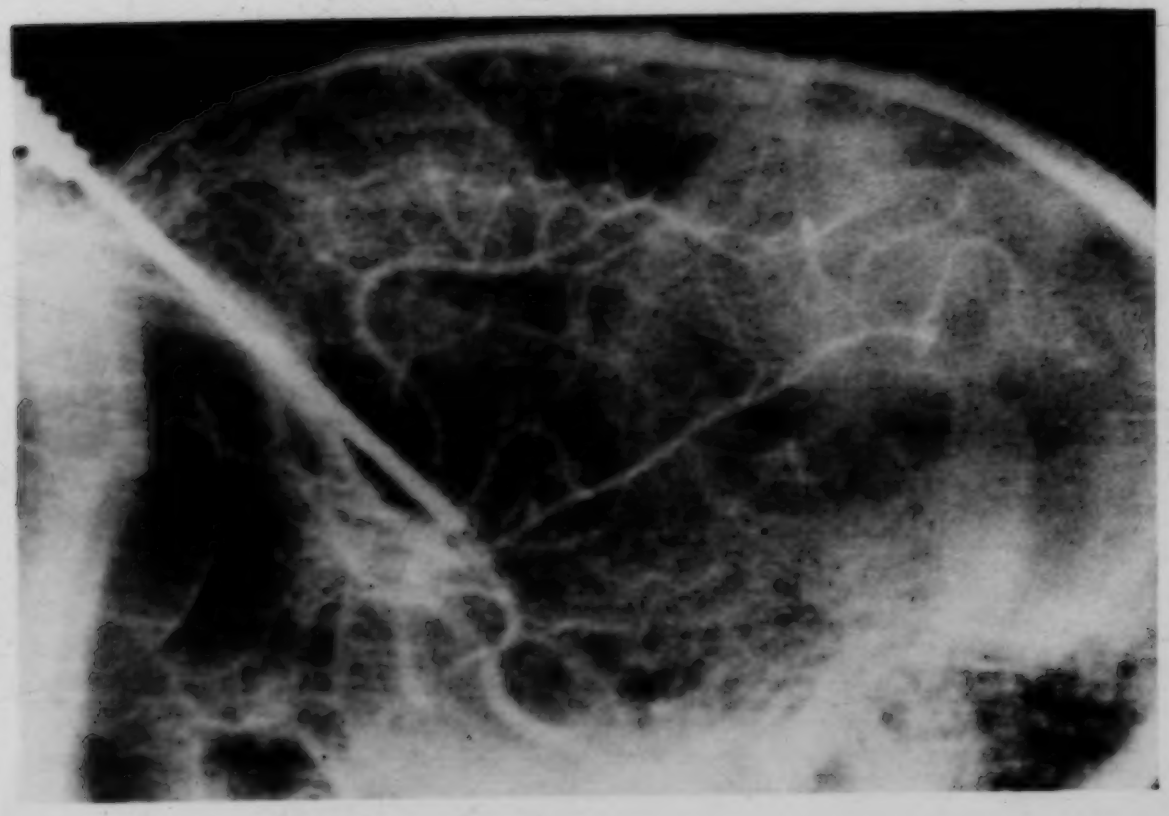
Four mL of whole blood was collected in 0.4 mL of acid citrate dextrose solution (NIH - solution 3) and centrifuged for 10 min at 800 rpm to produce platelet-rich plasma. This plasma was separated and re-centrifuged at 2000 rpm for 10 min to form a platelet concentrate. The concentrate was suspended in 0.2 - 0.3 mL of plasma and contained 1.0 - 1.2 x 10^6 platelets / mL concentrate. Platelet concentrate was added to 1.0 - 1.5 mL/kg fresh, autologous blood and administered immediately as the SAH. Periodically, 0.5 mL samples of this injectate were allowed to stand in the laboratory to confirm that the mixture would coagulate.

The SAH

A cranial twist-drill hole (1.5 mm in diameter), 0.5 to 1.0 cm dorsal to the nasion, was drilled in all animals prior to subarachnoid injections. Hemostasis was achieved with bone wax application and a screw device was placed in the defect.

A circumferentially bevelled #20-gauge spinal needle was advanced under fluoroscopy (Phillips Ltd. - Holland), through the midline frontal twist-drill hole, along the floor of the anterior fossa to 0.5 - 1.0 mm beyond the anterior clinoid processes. When the tip of the needle had entered the chiasmatic cistern, the needle was secured to the skull by the screw device (Fig 3). In all cases, there was drainage of

Fig 3: Lateral angiogram showing #20-gauge spinal needle secured to the skull by a screw device. The tip of mid-line needle is in the chiasmatic cistern with the body along the floor of the anterior fossa.



1.0 - 1.5 ml of CSF prior to the induction of SAH.

The animals were divided into 2 groups, a small hemorrhage group of 10 monkeys (1.0 mL/kg) and a large hemorrhage group (1.2 to 1.5 mL/kg) of 12 monkeys. In the small hemorrhage animals, the SAH was placed over 5 minutes, while in the large hemorrhage group, the first mL/kg was placed over the first 5 minutes and the remainder over 15 minutes. The injection was terminated if a Cushing response developed, defined as an increase in pre-SAH BP of more than 25%, or if the pupils became dilated and unresponsive to light.

Early postoperative follow-up

After SAH, the animals were allowed to stabilize for 1 hour during which time BP and arterial blood gases were allowed to return to pre-SAH values. Carotid angiography was repeated following which anaesthesia was discontinued and the animals were permitted to breathe 100% oxygen. Paralysis was reversed with neostigmine (Prostigmin R - Hoffman - La Roche Ltd. - Vaudrevil, P.Q.) (0.04-0.06 mg/kg) IV and dl-hyoscyamine (Atropine R - Glaxo, Toronto, Ontario) (0.02 0.03 mg/kg) IV.

Upon extubation and recovery from anaesthesia, the animals were assigned to a treatment or control group as determined by a restricted randomization assigned before the project began. The treatment group was given reserpine 0.013 mg/kg

intramuscularly (IM) and kanamycin 50 mg/kg injected into an orange sliver, for oral consumption (PO). The control animals received IM and PO saline. All animals received the PO and IM administrations on a daily basis as confirmed by a double-check sign-off system. In no case did the animals refuse to consume the orange sliver containing the kanamycin or placebo. No obvious clinical signs allowed distinction between the treatment and control animals. Therapy was continued to Day 7 at which time the animals were re-evaluated.

Neurological grading system

Neurological examination was carried out on each monkey before experimentation. Of particular interest was the level of consciousness, symmetrical extremity strength and mobility, pupillary responses to light and corneal reflexes. A 5-point neurological grading system (Petruk, 1976) was applied (Table 8) on Day 0 and daily thereafter up to Day 7.

Day 7 follow-up

On Day 7, each animal was returned to the laboratory and following complete neurological examination, anaesthesia was reinduced as previously described. The left common carotid artery was again cannulated at 0.5 cm distal to the first purse-string site. Arterial blood gases and BP were restabilized to day 0 values, blood for serotonin estimation was collected, and carotid angiography was repeated.

Table 8: Neurological grading system (Petruk, 1976)

NEUROLOGICAL GRADING SYSTEM

GRADE 1: Alert, active, vocal with no evidence of neurological abnormalities.

GRADE 2: Mildly obtunded, lethargic, no obvious neurological deficit.

GRADE 3: Moderately obtunded, but arousable by stimulation (sound, touch or pain). Demonstrate neurological deficit (i.e. non-reactive pupil, mono or hemi-paresis).

GRADE 4: Severely obtunded, responsive only to deep painful stimuli.

GRADE 5: Moribund. Grade 4 status with unstable vital signs.

Pathology

Following the completion of all measurements on Day 7 all animals were humanely sacrificed by pentobarbital overdose and postmortem examination of the brain was performed to confirm the position of the intracranial hemorrhage. Animals were considered nonevaluable and eliminated from the study if any significant blood collection existed in the subdural space. Significant blood collection was assumed if there was any demonstrable hematoma in the subdural space. It was anticipated that little, if any, subarachnoid blood would still be present at 7 days post-SAH, however diffuse cortical or basal pigmentation was used as an indicator of reabsorbed subarachnoid blood.

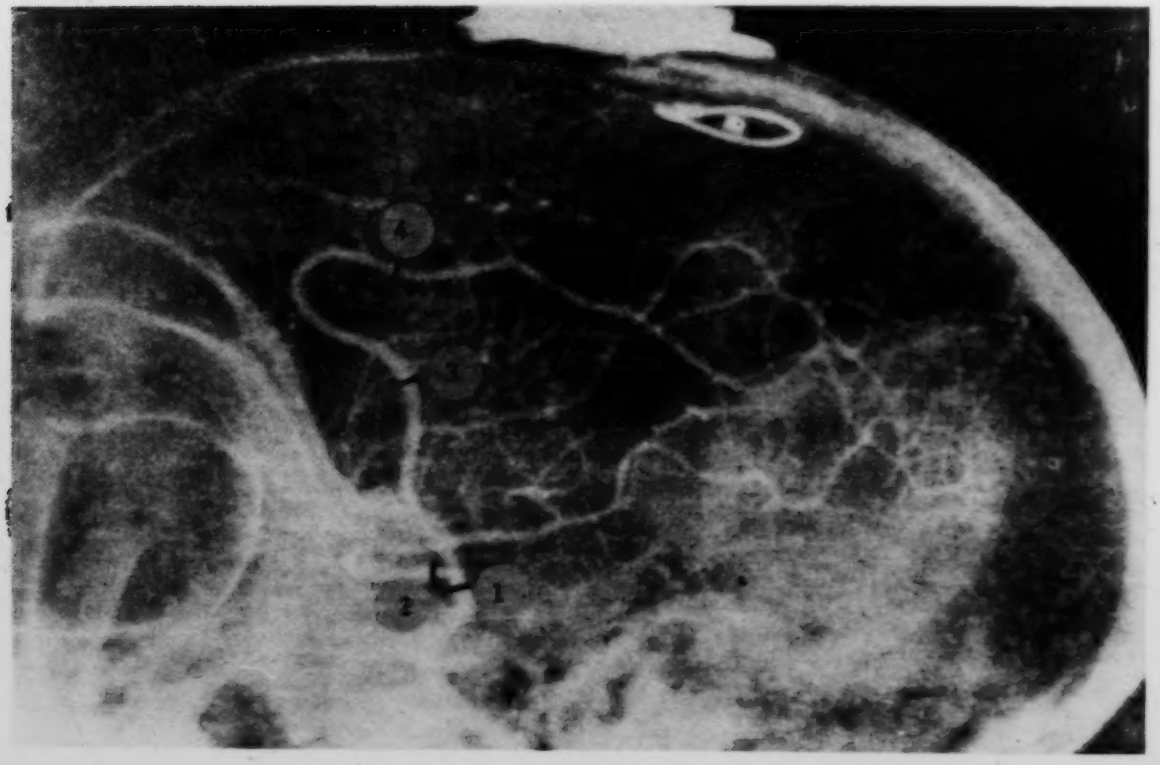
Carotid angiography

Lateral and oblique angiograms were obtained on Day 0 (pre-SAH and 1 hour post-SAH) and Day 7. Magnification factors were kept constant by a radiopaque control device used to correct for variation in film, screen, exposure and developing factors. Vessels were measured at 4 fixed points: the internal carotid artery just above the posterior communicating artery (ICA); the proximal middle cerebral artery (MCA); the proximal pericallosal artery (PPC) and the distal pericallosal artery (DPC). (Fig 4).

Using an optical measurement system, an independent observer unaware of the animals' treatment group measured the

Fig 4: Vessel measurements at 4 fixed points:

1. internal carotid artery just above the posterior communication artery.
2. proximal middle cerebral artery.
3. proximal pericallosal artery.
4. distal pericallosal artery.



4 arterial sites 6 times (24 measurements) pre-SAH, 1 hour post-SAH and at 7 days post-SAH, i.e. 72 measurements per animal. The mean was then calculated for each site. VSP was defined as >10% reduction of vessel caliber from the initial values and graded as follows: mild, 11 - 30%; moderate, 31 - 50%; and severe, >51%.

Serotonin analysis

Recognizing that serotonin in whole blood can be protected from degradation by using EDTA and ascorbic acid, Yuwiler et al (1970) reported, an accurate procedure for the determination of serotonin in whole blood. The assay used in this experiment followed this protocol. Reagents used in this assay are listed in Table 9.

Whole blood (2.5mL) was collected in EDTA-treated tubes on day 0 pre-SAH and day 7. All glass used in the assay and in the prepartion of reagents was deionized to obtain consistent results. Because cold storage of blood may result in up to 10% loss in serotonin content within 48 hours (Yuwiler et al, 1970), all assays were done within 8 hours of obtaining the sample. No effort was made to control the diet of the animals before determination of whole blood serotonin in view of Yuwiler's suggestion that dietary changes seemed to have little effect on blood serotonin levels. For example, he reported that one subject ingested three bananas (1 1/8 lb), a food known to be rich in serotonin, 1 hour before serotonin

Table 9: Serotonin analysis

REAGENTS USED IN SEROTONIN ANALYSIS

- YUWILER METHOD

1. NaCl 0.85%.
2. Ascorbic acid saturated with disodium ethylene dinitrito tetra acetate (EDTA) and KCl.
3. Dibasic potassium phosphate, 2 M saturated KCl and adjusted to pH 10.0 with freshly prepared concentrated KOH pellets.
4. n-Butanol saturated with water.
5. HCl-12N and 0.1N.
6. Cyclohexane.
7. Serotonin stock solution prepared by dissolving 11.5 mg of serotonin creatinine sulfate in 500 ml of water to give 10 ug of free base/ml.

determination. He found a variation of 0.003 ug/mL as compared to the 2 month average of 5 further determinations. Similarly, he noted that amongst his subjects, even after eating a hearty breakfast, there was no significant alteration from fasting serotonin levels. This point is of note in view of the fact that the vehicle used to carry the serotonin and antagonist kanamycin, or placebo, was an orange sliver.

Data analysis

Data for all monkeys was coded, entered into a computer, and edited. Descriptive statistics and frequencies were determined for each variable at each time period by group. Comparison between groups at entry was made using analysis of variance. The large and small hemorrhage categories were combined when it became apparent that no difference in the frequency or severity of the VSP was produced by variable SAH size. Analysis of covariance was used to compare the groups at the end of each time period, with the initial starting values of each variable being used as the covariate. The incidence of VSP and Cushing's response were analysed by the Fisher-Irwin test. A level of statistical significance for all tests of comparison or association was $P<0.05$.

EXPERIMENTAL RESULTS

Twenty-seven animals were studied. Three animals died within 3 hours of subarachnoid hemorrhage, two had extensive subarachnoid blood around the basal surfaces of the brain and one animal showed predominantly subdural and intracerebral hemorrhage. Two further animals were eliminated at postmortem on day 7 because of significant subdural hemorrhage which was easy to identify as a hematoma formation. The remaining 22 animals successfully completed the 7 day study. For analysis, animals were divided into four groups (Table 10).

Variables

Baseline analysis of the 4 groups, using the analysis of variance, showed no significant differences in pre-SAH control values (time 1) for body weight, BP, arterial blood gases or angiographically-measured vessel caliber in ICA, MCA, PPC, DPC (Table 11a, b). The neurological status of the 4 groups was also not significantly different by the Fisher-Irwin exact test.

Analyses of BP and arterial blood gases in all animals were carried out pre-SAH (time 2); 1 hour post-SAH, pre-angiography (time 3); and at 7 days post-SAH, pre-angiography (time 4). Table 12 provides the mean and standard errors of these variables in these 4 time frames, with the small and large hemorrhage animals combined as a group.

Table 10: Animal treatment group

ANIMAL TREATMENT GROUPS

GROUP 1 - Small hemorrhage - control (n=5)

GROUP 2 - Small hemorrhage - treatment (n=5)

GROUP 3 - Large hemorrhage - control (n=6)

GROUP 4 - Large hemorrhage - treatment (n=6)

Table 11 a. Control values (baseline) for measured and observed variables (x ± S.E.)

GROUP	BODY WEIGHT (kg)	MEAN BP (mmHg)	PaO_2 (mmHg)	$PaCO_2$ (mmHg)
1. Small Hemorrhage Not treated (N=5)	3.2 ± 0.2	100 ± 4	182 ± 16	37 ± 2
2. Small Hemorrhage Treated (N=5)	3.1 ± 0.3	95 ± 5	180 ± 13	40 ± 1
3. Large Hemorrhage Not treated (N=6)	3.2 ± 0.2	88 ± 6	210 ± 10	38 ± 1
4. Large Hemorrhage Treated (N=6)	3.5 ± 0.1	86 ± 2	195 ± 10	40 ± 1

Table 11 b. Control values (baseline) for measured and observed variables (X ± S.E.)

		VESSEL CALIBRE (mm)				
						INJECTATE
GROUP	pH	ICA	MCA	PPC	DPC	VOL mL /kg
1. Small Hemorrhage Not treated (N=5)	7.41±0.1	1.0±0.1	0.7±0.1	0.8±0.1	0.7±0.1	1.0±0
2. Small Hemorrhage Treated (N=5)	7.42±0.1	0.9±0.1	0.6±0.1	0.8±0.1	0.7±0.1	1.0±0
3. Large Hemorrhage Not Treated N=6)	7.46±0.1	0.9±0.1	0.6±0.1	0.7±0.2	0.7±0.1	1.4±0.1
4. Large Hemorrhage Treated (N=6)	7.50±0.1	0.8±0.1	0.6±0.1	0.7±0.1	0.6±0.1	1.3±0.1

Table 12. Minor variables over time in small and large hemorrhage groups combined (X ± S.E.)

		BP (mmHg)	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	pH
Small SAH animals (Groups 1&2 combined)					
Time	1	97 ± 10	39 ± 3	181 ± 31	7.42 ± 0.01
	2	103 ± 8	38 ± 2	175 ± 29	7.41 ± 0.02
	3	102 ± 12	39 ± 4	183 ± 32	7.37 ± 0.01
	4	89 ± 10	37 ± 4	182 ± 40	7.45 ± 0.01
Large SAH animals (Groups 3&4 combined)					
	1	87 ± 10	39 ± 4	202 ± 25	7.48 ± 0.01
	2	93 ± 12	37 ± 2	198 ± 32	7.46 ± 0.02
	3	94 ± 8	36 ± 4	189 ± 40	7.46 ± 0.03
	4	91 ± 10	37 ± 2	205 ± 39	7.48 ± 0.02

The volume of subarachnoid hemorrhage injectate (Table 11b) was significantly different ($P<0.01$) in groups 1 and 2 combined versus groups 3 and 4 combined.

There was no significant difference between groups 1 versus 2 or groups 3 versus 4 for BP, PO_2, PCO_2 or PH. Groups 3 and 4 combined demonstrated more alkalemia than groups 1 and 2 combined ($p < 0.001$). This alkalemia lacked satisfactory explanation and was not on the basis of hyperventilation in the large hemorrhage animals in view of the similar $PaCO_2$ levels. There was no significant difference between groups 3 and 4 combined versus groups 1 and 2 combined for BP, PO_2 or PCO_2 (analysis of covariance).

Finally, there was no consistent change in BP over time for any of the 4 groups, with the exception of variable increases in blood pressure during induction of SAH, seen in virtually all animals.

Serotonin values

Analysis of serotonin estimations carried out pre-SAH showed no significant difference between any of the 4 groups (Fig 5). However, there was a significant difference in the serotonin level at 7 days between the treated and control small-SAH animals ($p<0.01$) and the treated and control large-SAH animals ($p < 0.05$). Figure 6 shows the change in serotonin levels over 7 days in combined groups 2 and 4 (treatment) and 1 and 3 (control).

Fig. 5: Baseline serotonin values. No significant difference in the four groups.

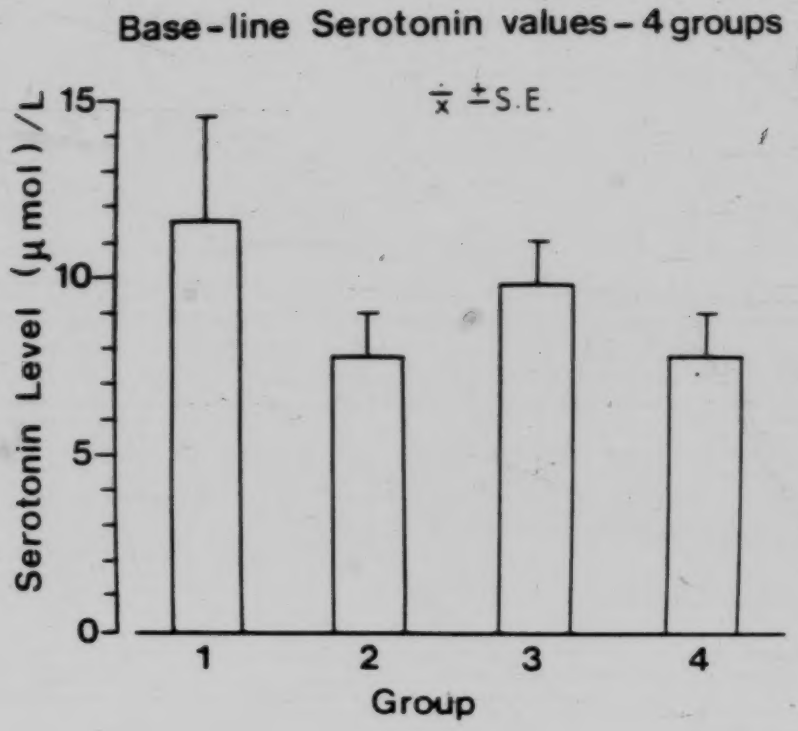
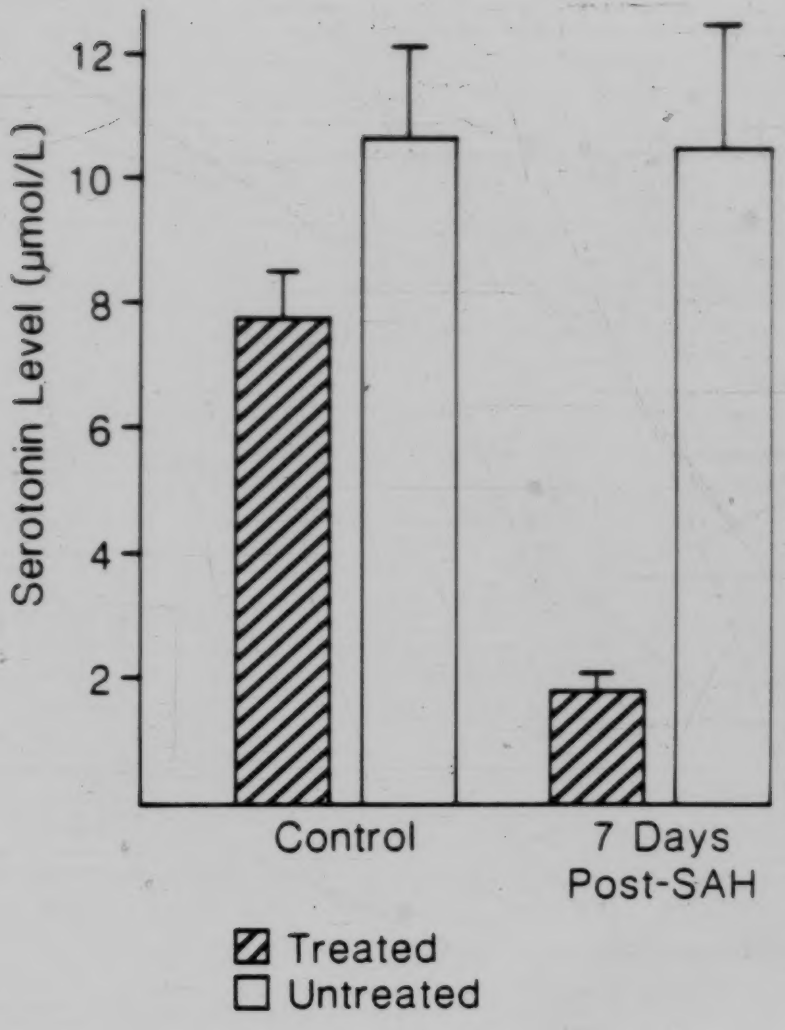


Fig. 6: Serotonin levels ($\bar{X} \pm$ S.E.) day 0 versus day 7 for treated animals (group 2 & 4) and control animals (group 1 & 3).



Serotonin levels were 19 and 26% of control in the small and large hemorrhage treated groups, respectively (Table 13).

Vasospasm

Defining VSP as greater than 10% reduction of vessel caliber from the control values, grade 1 (10-30%) or grade 2 (31-50%) VSP occurred in 77% of all animals at 1 hour post-SAH and 73% of animals at 7 days. Table 14 demonstrates the percent reduction in vessel caliber of the maximally constricted vessel and VSP grade for each animal in the 4 groups at day 0 (1 hour post-SAH) and day 7 (post-SAH). Serotonin pre- and post-ratios for each animal are included.

VSP was distributed through the 4 groups (Table 15). There was no significantly different frequency in either of:

1. The small versus the large hemorrhage groups,
   or more importantly,

2. The treatment versus the nontreatment groups.

Figure 7 shows the average vessel caliber (6 measurements per vessel) at all measured (4 per angiogram) sites, pre-SAH and 7 days post-SAH for all treated and control animals (48 measurements per animal). Figure 8 illustrates the percent change in vessel calibre, of the vessels showing the greatest degree of VSP in each animal that developed VSP (n=16), in the combined treated and untreated groups.

Table 13: Serotonin levels at day 0 and 7 for all groups. Values are also expressed at % baseline and pre:post treatment ratios.

	Day 0 (Baseline) (umol/L)	Day 7	%Baseline	Pre:Post-serotonin ratio
Small-SAH Groups (1&2)				
control	11 ± 3.0	12.5 ± 3.7	100	1.0 ± 0.1
treatment	7 ± 0.4	1.5 ± 0.3	19	6.1 ± 1.5
	P = N.S.	P < 0.01		
Large-SAH Groups (3&4)				
control	9.8 ± 1.2	8.8 ± 2.0	90	2.6 ± 0.6
treatment	7.8 ± 1.3	2.1 ± 0.5	26	4.1 ± 1.7
	P = N.S.	P < 0.05		

Table 14: Percent reduction in vessel calibre (vessel with greatest degree of VSP) and VSP grade for each animal in the 4 groups on day 0 (post-SAH) and day 7 (post-SAH). Serotonin pre:post ratio for each animal included.

Group	Animal #	DAY 0		DAY 7		
		1 hr post-SAH % reduction Vessel Caliber	Grade VSP	Post-SAH %reduction Vessel Caliber	Grade VSP	Serotonin Pre:Post ratio
1 (Small-SAH Control)	216	12.5	1	0	0	0.8
	223	13.0	1	15.2	1	0.9
	225	15.5	1	0	0	0.8
	228	15.0	1	13.0	1	1.2
	230	13.1	1	37.7	2	1.1
2 (Small-SAH treatment)	214	35.0	2	23.8	1	4.9
	215	19.0	1	27.8	1	11.8
	218	34.5	2	27.3	1	3.5
	220	0	0	12.0	1	4.5
	221	33.8	2	28.6	1	5.7
3 (Large-SAH Control)	235	23.2	1	0	0	1.1
	236	40.2	2	28.7	1	0.7
	237	0	0	0	0	0.7
	240	28.6	1	24.0	1	0.7
	243	0	0	0	0	1.5
	246	13.4	1	17.4	1	11.1
4 (Large-SAH Treatment)	238	21.1	1	40.0	2	6.3
	239	32.8	2	17.9	1	3.1
	242	15.5	1	11.9	1	3.1
	244	22.6	1	20.2	1	5.5
	247	0	0	20.3	1	3.2
	248	0	0	0	0	3.5

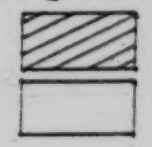
Table 15: Distribution of VSP over 4 groups.

PERCENTAGE OF MONKEYS IN EACH VASOSPASM* GRADE AT 7 DAYS

GROUP	NONE	MILD GRADE	MODERATE GRADE	MILD & MODERATE GRADE
SMALL HEMORRHAGE NOT TREATED	40	20	40	60
SMALL HEMORRHAGE TREATED	0	100	0	100
LARGE HEMORRHAGE NOT TREATED	50	50	0	50
LARGE HEMORRHAGE TREATED	17	67	17	84

*THIS WAS THE GRADE OF THE INDIVIDUAL VESSEL SITE OF THE 4 MEASURED SHOWING THE GREATEST DEGREE OF VSP. GRADE: MILD = -11 - -30%, MODERATE = -31 - -50%.

Fig. 7: Average vessel caliber of all measurement sites ($\bar{X} \pm$ S.E.)



treated groups combined (2&4)

control groups combined (1&3)

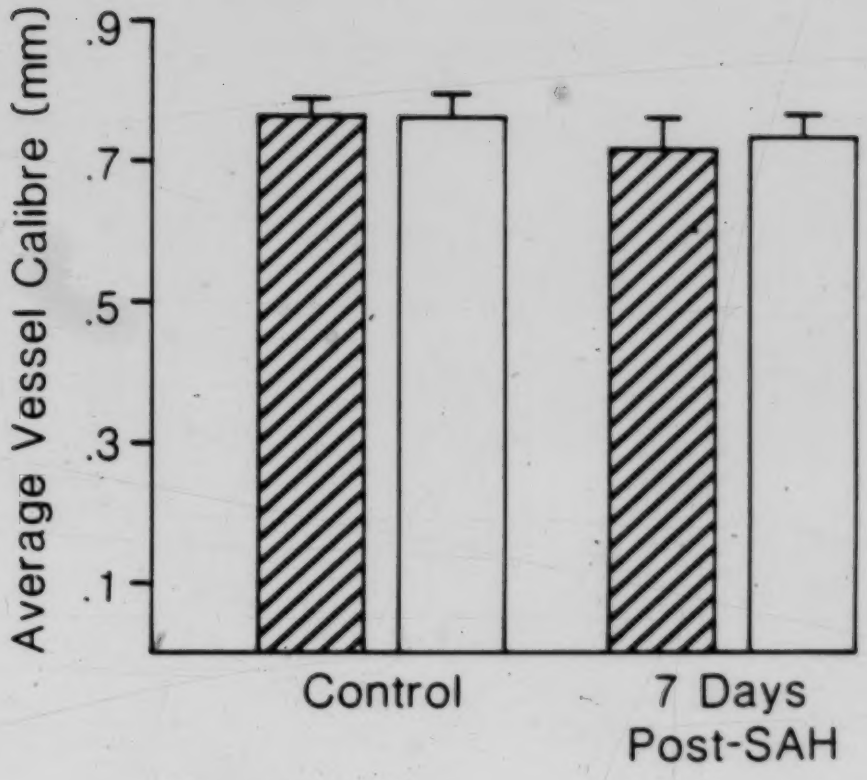


Fig. 8: Percent change in vessel calibre, of the vessels showing the greatest degree of VSP in each animal. Both control (groups 1&3) & both treated (groups 2&3) groups are combined.

Sixteen of 22 animals developed VSP at 7 days.
($\bar{x} \pm$ S.E.)

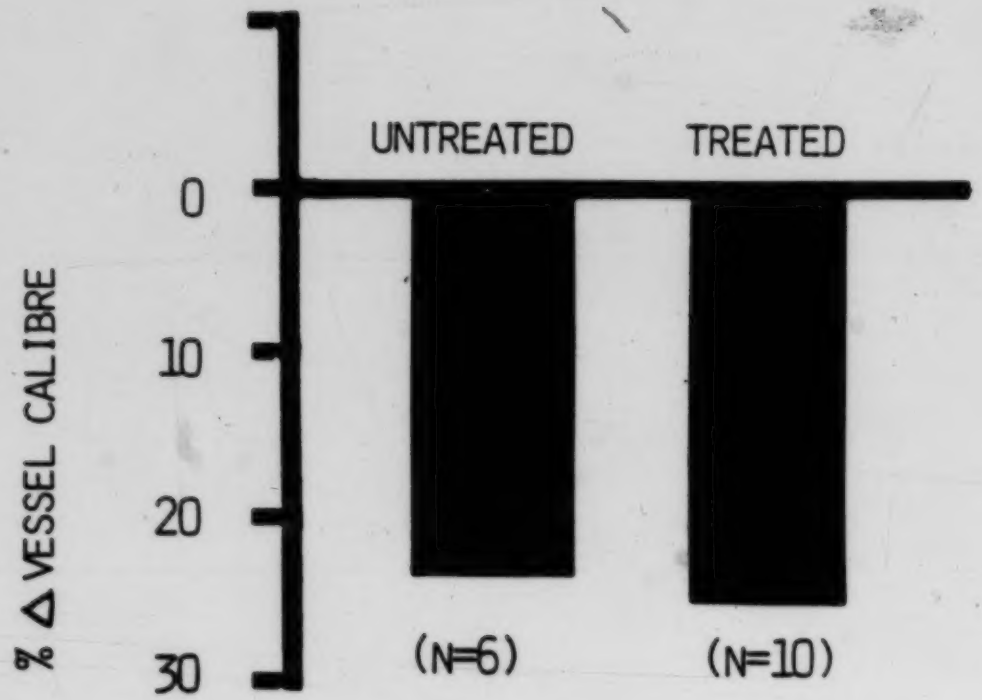


Figure 9a & b demonstrates the angiographic appearance of an animal showing moderate vasospasm.

Neurological examination

Neurological deterioration developed in 3 animals on day 0 (grade 2). On day 7 follow-up, 1 of these animals was graded as having no VSP, 1 as mild VSP and 1 as moderate VSP. A fourth animal had a depressed level of consciousness for 48 hours, characterized by only eye opening and limb flexion in response to pain (grade 3). This resolved on day 3 and was not associated with further neurological deficits. Cerebral angiograms on day 7 showed no significant VSP but significant brain swelling, believed to represent cerebral edema, was present on postmortem examination.

Postmortem examination

At the completion of each experiment, animals were humanely sacrificed using barbiturate overdose. The brains were removed intact, to confirm the site of the previous SAH. It was not possible 7 days post-SAH to estimate satisfactorily the size of the SAH, in that the majority of subarachnoid blood was reabsorbed by this time, leaving a persistent diffuse hemorrhagic pigmentation over the base of the brain and usually the cortical surfaces. On the other hand, significant subdural hematoma was easily identifiable as a localized blood collection. Autopsy examination of the brain

Fig. 9a: Cerebral angiography in an animal day 0 - pre-SAH

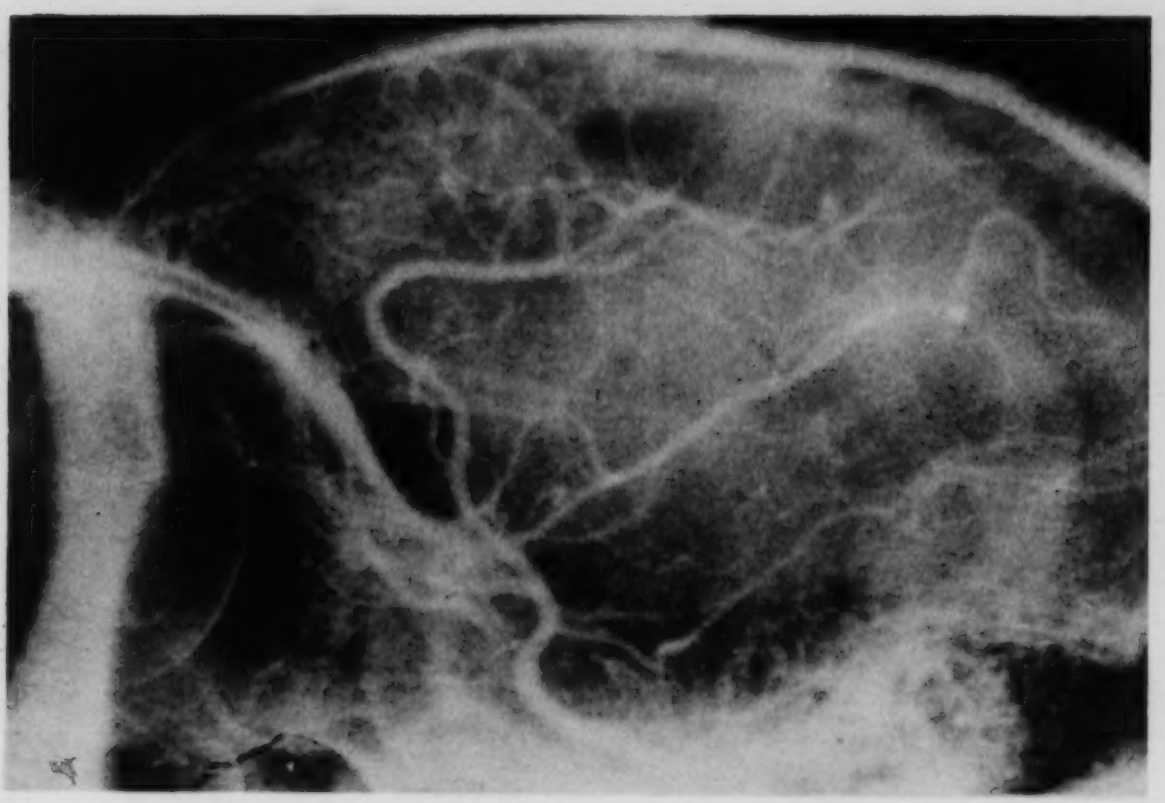
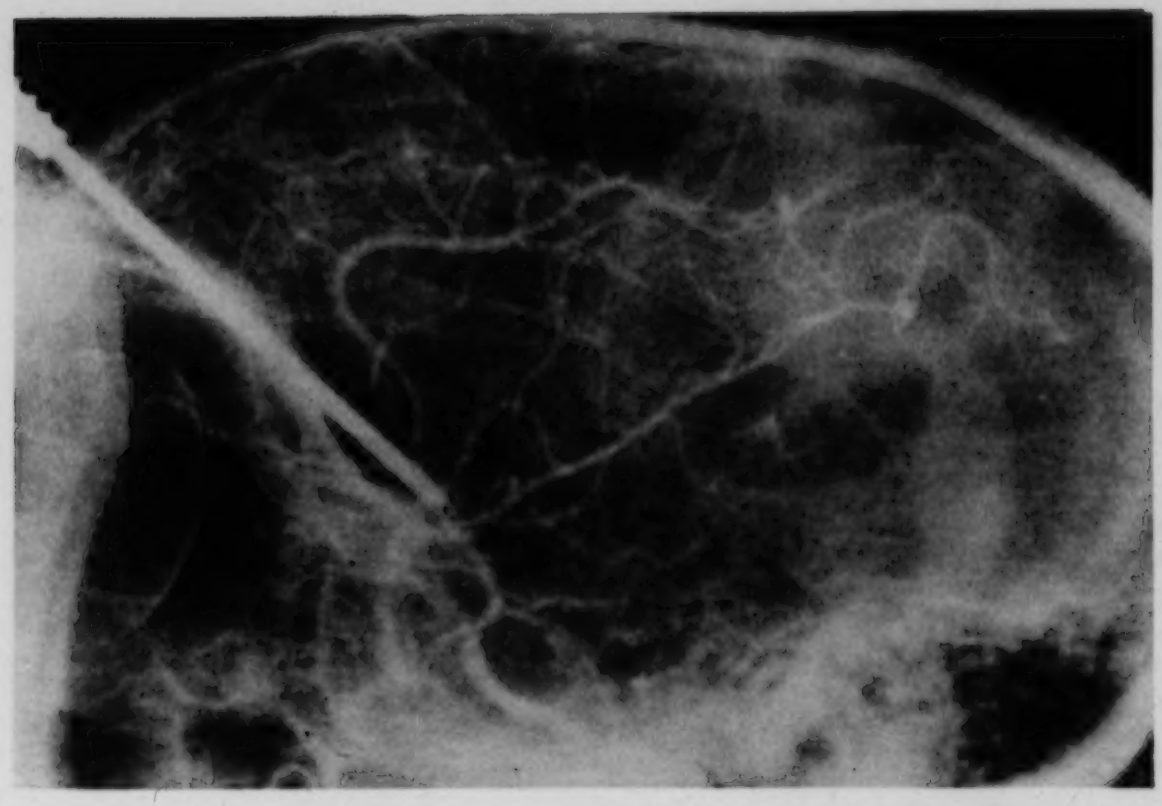


Fig. 9b: Cerebral angiography in same animal as 9(a) 1 hour post-SAH, demonstrating significant VSP in ICA, MCA, PPC, DPC.



was used to exclude 2 animals on day 7 because of significant subdural blood. Figure 10 demonstrates an acute SAH in an animal dying within 3 hours of the insult. Figure 11 shows pigmentation at the base of the brain 7 days post-SAH.

Cushing response

Using a definition of Cushing's response as a peak in blood pressure greater than 25% above values immediately pre-SAH, 9 animals developed the response (3 of 10 animals from the small hemorrhage groups, and 6 of 12 animals from the large hemorrhage groups). There was a significant relationship between the development of Cushing's response and subsequent VSP at 1 hour ($p < 0.05$) but not at 7 days (Fisher-Irwin exact test).

There was no significant difference in the occurrence of Cushing's responses in the small versus the large hemorrhage groups, nor between the treated and untreated groups (Table 16) (Fisher-Irwin exact test).

Fig. 10: Acute subarachnoid hemorrhage

Fig. 11: Post-mortem appearance of SAH - day 7

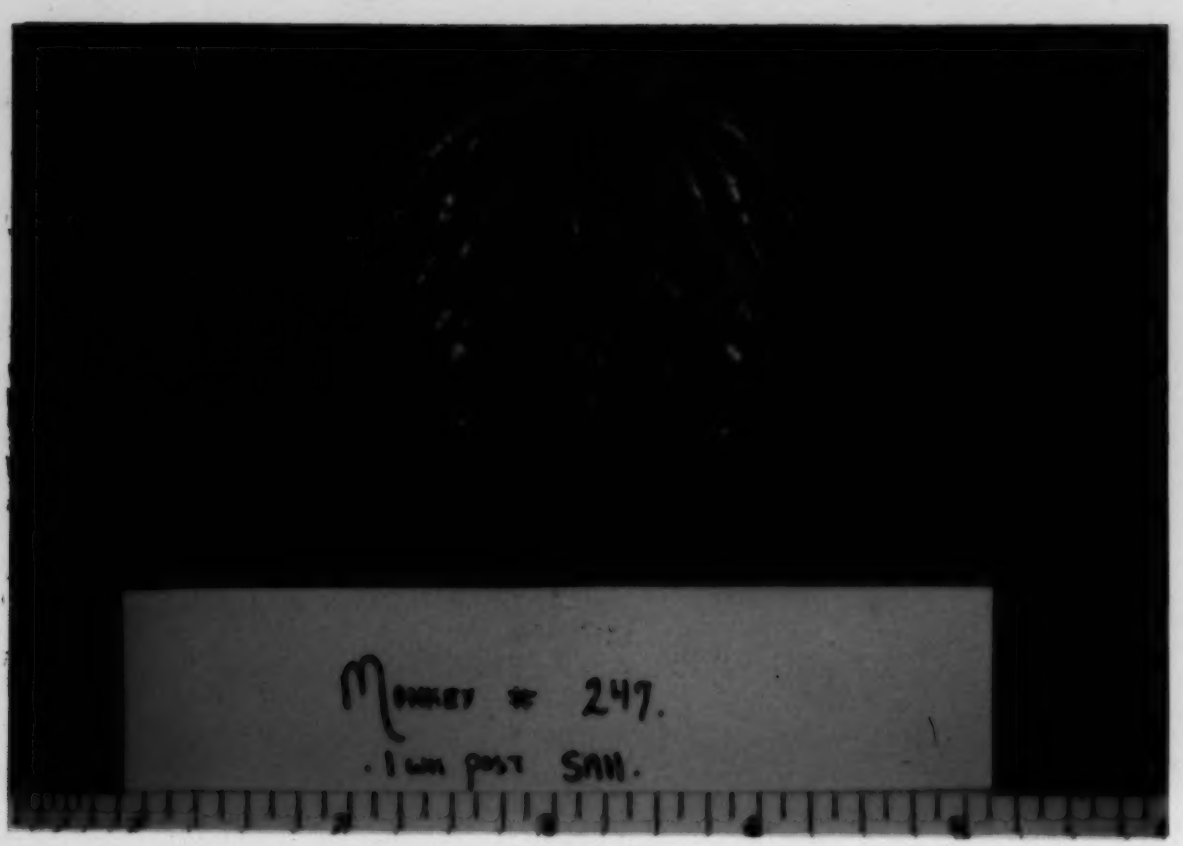


Table 16: Cushing response at time of SAH

CUSHING RESPONSES AT TIME OF SAH

	Small groups	Large groups
No	7	6
Yes	3	6

CHAPTER 3

CONCLUSIONS AND DISCUSSION

This study was fostered by uncertainties regarding the role of serotonin as the mediator for cerebral VSP following SAH. In vitro and in vivo studies have provided arguments for and against serotonin as the putative spasmogen. What useful contributions did this study add to the vast, already available literature on the subject of cerebral VSP associated with SAH?

The following conclusions were drawn:

1. Introduction of a SAH consisting of autologous blood and platelet concentrate into the chiasmatic cistern results in angiographic cerebral VSP in a significant proportion of animals at 1 hour (77%) and at 7 days (73%).

2. Cerebral VSP, in this model, was no greater in frequency or severity if small SAH (1 mL/kg) as opposed to large SAH (1.2-1.5 mL/kg was produced.

3. Reserpine and kanamycin, in the dosage used, significantly lowered serotonin levels over a 7 day period.

4. Treatment with reserpine and kanamycin, following SAH, did not reduce the frequency or severity of cerebral VSP at 7 days despite the demonstrable reduction of blood serotonin levels.

5. In this model, delayed ischemic deficits did not occur in association with VSP.

6. Though the Cushing response at the time of SAH appeared to influence the development of early (1 hour - SAH) VSP, it bore no relationship to the development of VSP at 7 days.

In short, the objectives outlined on page 53 were fulfilled by this experiment and the hypotheses as stated were resolved. However, each of the conclusions deserve further consideration and comment.

#1) The development of VSP in 77 and 73% of animals at 1 hour and 7 days, respectively, supports the efficacy of the model used. Working independently with the same species and model Espinosa et al (1984) showed quite similar results. Sixty percent and 67% of a small/medium-SAH group demonstrated VSP at 1 hour and 7, days respectively. In a large-SAH group the frequency of VSP was 100 and 88% at 1 hour and 7 days respectively. The fact that there was a similar occurrence of VSP in this work and that of Espinosa, questions the role of the platelet in VSP. Platelets were added to autologous blood to accentuate any VSP which might result from vasoactive substances within the platelets.

Being able to demonstrate VSP at 7 days provided an experimental model with which to judge the efficacy of reserpine and kanamycin.

#2) Animals were divided into both small and large SAH groups under the presumption that the frequency and severity of VSP would correlate directly with hemorrhage size. In this experiment no such correlation was found, despite a statistically significant difference in injectate volume between both sets of animals. This would appear to be in conflict with Espinosa (1985) who showed that the size of SAH on computed tomography (CT) influenced the frequency and severity of VSP. This discrepancy can be reconciled in that the size of hemorrhage in that study was determined by CT measurement of blood clot thickness, not the volume of injectate. In fact, in those experiments the CT size of hemorrhage often bore no relationship to the quantity of blood instilled. This points to a flaw in the model seen in both this work and that of Espinosa, namely, that the instillation of blood into the chiasmatic cistern does not guarantee subarachnoid placement of the entire injectate. Assuming that fluroscopy control and recovery of CSF prior to SAH are evidence of correct needle placement, explanations for this discrepancy include extravasation from the needle tip back through the dural defect. An increased pressure development within the cistern during or following injection similarly would result in back-flow to the subdural space.

#3) As expected reserpine and kanamycin significantly lowered serotonin levels over the 7 day period. The proportionate dose used in the human studies of Zervas et al

(1979) determined the dose and schedule for this study. One might have expected that reserpine would have lowered the blood pressure in the treated animals. Watts (1977), who treated some animals in his series with reserpine 0.1 mg/kg, reported no blood pressure data. For those dogs that received reserpine (0.1 mg/kg) in the experiments of Zervas et al (1973), marked differences in blood pressure were reported between the treated and untreated groups. Finally, in the human experiments performed by Zervas et al (1979) no blood pressure data was included but patients only received reserpine 0.2 mg subcutaneously q.i.d. Assuming 60 kg mean weight for the patients, in that weight was not reported, that provided for approximately 0.013 mg/kg dose. Given the choice of 0.1 mg/kg or 0.013 mg/kg from the cited studies,the latter was chosen for this experiment not only to duplicate the human study but to lessen the likelihood that the dose would adversely affect blood pressure and in so doing complicate the interpretation of the angiographic data.

#4) The central issue of these experiments was to determine if the treatment with anti-serotonin drugs would alter the development of VSP. In short, it did not. On face value this suggests that such drug therapy in humans is not efficacious if prescribed in the post-ictal period. Even if medications which lower serotonin levels or oppose its effects have been shown in vitro to prevent or reverse the vasoconstriction, this model which reliably demonstrated VSP,

failed to demonstrate that reserpine and kanamycin "after the fact" alter the course of VSP.

#5) It is discouraging that so few animals showed any neurological signs associated with the insult. Grade 2 assessment of 3 animals on day 0 could have been related to the experimental manipulation, or anaesthetic. VSP grades 0, 1 and 2 were found in these animals at 7 days, allowing no conclusive association. The one animal which developed a grade 3 assessment had no hemiparesis or focal deficits. The altered consciousness resolved by the third postoperative day and, therefore, if related to acute VSP is not analagous to the human time course. Of importance, there was no VSP at 7 days and at autopsy there was brain swelling likely related to cerebral edema. It is well appreciated that intracranial hypertension is seen in both animal models (Petruk, 1976; Kamiya et al 1983) and humans. This may well have produced the altered consciousness in this animal and predisposed to the brain swelling witnessed at 7 days. Despite the altered state of consciousness this animal still consumed the orange sliver containing kanamycin or its placebo as evidenced by the double check-off system.

#6) The Cushing's response did not relate to VSP at 7 days. The pathophysiology associated with the Cushing's response is associated with intracranial pressure (ICP) increase and is probably related to transtentorial pressure gradients (Fitch and McDowall, 1977; Fitch et al 1977). Not

all of the animals developed this reflex, which doubtless results from the variability in pressure-volume characteristics that occur from animal to animal, as indeed from human to human. Stornelli and French (1969) suggested that diffuse VSP was only associated with severely increased ICP. Farrar (1975) reviewed the role of ICP in chronic cerebral VSP and suggested that diffuse arterial narrowing and reduced flow occurred with transmural pressures below 50 mmHg. Whatever the relationship between ICP and VSP, the in vitro work of Farrar has not been reproduced in in vivo VSP experiments (Fraser, 1980).

Criticisms

Despite the fulfillment of objectives and resolution of the hypotheses, criticisms of the experimental design, methods and results are warranted.

In similar experiments Espinosa continued the studies for up to 21 days (1985). The maximum percentage of vessels in spasm in a large-SAH group was greatest on day 0 and 7. Though VSP was "chronic" in this experiment, in that it was demonstrable at 7 days, it is possible that a longer period of study, possibly up to 21 days post-SAH would have delineated some difference accrued from therapy in the groups.

As stated, though VSP occurred in a significant portion of all animals, none developed delayed ischemic deficits that are typical of the human disorder. It is difficult to apply

what is found in the laboratory to the human experience, as a result of this (Noseworthy, 1984 - see reviewer's comments, pg 197). Studies by most other investigators working in this field have also been open to this appropriate criticism (Fraser, 1980). Not only does early VSP seen in animals within a few hours of SAH not have a human analogy, but when chronic VSP can be demonstrated in animals there is often no clinical correlation with focal deficits as in humans.

Macroscopically the circulation of the animals appears comparable to that of the human. Is collateral circulation different? Espinosa (1984) did not see delayed ischemic deficits associated with significant VSP either, except in a single animal. Collateral circulation was seen in the animals and it was maximal when VSP was most severe, possibly preventing distal ischemia. Of course, microscopic variability in circulations, or variability in the propensity to develop collateral flow could well be very different in cynomolgus monkeys as opposed to humans.

An unanswered question is whether there are unique factors associated with SAH emanating from an abnormal vessel with saccular aneurysm, as opposed to SAH induced by introduction of autologous blood or by puncture or avulsion of a normal vessel. This appears unlikely, however it is difficult to explain why some authors (Fraser, 1980) have not seen the sequence of VSP associated with coincidental massive SAH occurring during neurosurgical procedures. If unique

factors do exist in the vessel walls of saccular aneurysms they have yet to be identified.

Furthermore, it is distressing that many drugs have been shown to reverse blood-induced vasoconstriction in animals, but none has so far survived the test of time in humans (Wilkins, 1980).

Though an argument can be made that this experiment does not duplicate the prevention of post-operative VSP by pre-operative use of reserpine and kanamycin, it was not intended to do so. This experiment was intended to duplicate the work of others who have suggested that pre-operative VSP is reduced by these drugs administered following SAH. To my mind it is debatable that pre- and post-operative VSP should be considered different entities. Though it is conceivable that post-operative VSP may occur within hours of aneurysmal surgery, Saito et al (1977) have reported that there is little difference in the peak incidence of post-ictal VSP (7.6 ± 2.5 days) versus post-operative VSP (6.7 ± 1.6 days). While on the one hand SAH from sources other than ruptured saccular aneuryms does not appear to be associated with frequently recognizable VSP, surgical repair of aneuryms with virtually bloodless fields may still result in severe post-operative deficits. One might conclude from this that blood in the subarachnoid space is not the essential causative factor in human VSP. Alternatively, blood from the original SAH, whether or not it causes post-ictal pre-operative VSP may

still induce changes in cerebral vessels that "sensitize" them to the development of post-operative VSP. An obvious question that comes from this is whether patients subjected to early aneurysmal surgery before the development of post-ictal VSP, have a higher or lower incidence of post-operative VSP. The available evidence (Wheelock, 1983) suggests that the incidence of VSP is at least no higher if the surgery is early.

The significant criticism of this study relates to the method of carotid cannulation for performing cerebral angiography. Though this maneuver was performed in both the treated and control animals, it is discomforting to manipulate a proximal vessel and draw conclusions from distal vessels. Furthermore, one is only given the opportunity to study one-half of the anterior circulation of each animal.

This study could not have occurred without the capacity of the animal used, to undergo the anaesthesia, surgery and SAH, and subsequently to recover and be assessable for 7 days. During this time there was never any question of the consumption of food containing kanamycin or placebo.

Finally, the strength of this study is that the randomization process to treatment or control, the blinding methods for treatment, serotonin estimation and vessel caliber measurements, left no clue as to the potential results until every experiment had been performed and all data was available.

Future Considerations and Speculation

This model helped to answer a series of specific questions satisfactorily but has now outlived its usefulness. Obvious improvements in approach would result if carotid artery surgery could be avoided and anterior angiography could be bilateral. Such changes in this laboratory have already resulted in substantial improvements (Espinosa, 1984).

This experiment did not answer the 5 questions posed on pages 5&6, dealing with recognized peculiarities in humans, as opposed to in vivo animal VSP. These questions must be answered.

Years of painstaking research by investigators dedicated to studying the pathophysiology and treatment of VSP has led to a multiplicity of in vitro and in vivo animal models, using the application of various vasoactive substances, and/or their inhibitors, to make observations on cerebral angiographic VSP. An entirely satisfactory model to mimic the human incidence, onset and course of VSP, after aneurysm rupture remains to be developed.

This conundrum could result in the proliferation of patient clinical trials directed at the use of many agents known to alter smooth muscle contractility. An optimistic speculation on the value of this eventuality could be that a "short-cut", to finding ideal agents to treat or prevent human VSP, may be short-coming. Alternatively, a pessimistic side

to a potential proliferation or drug trials could be a less critical exposure, of a number of vasoactive agents or their blockers, to patients that might otherwise have avoided them. The ideal animal model, therefore, should remain a paramount goal for investigators in this field.

It is highly probable that the cause of post-ictal VSP is multifactorial and that once there is propulsion of blood into the subarachnoid space that several mediators set the stage for subsequent vascular constriction. If the final common pathway or factors that induce or sustain vascular smooth muscle contraction can be found, blocked or reversed, in in vitro or subsequently in vivo studies, there will be a population of patients available for study and help in an effort to prevent or reverse this devastating event.

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